Primary hrHPV testing in cervical screening: the arguments

Nicole Bulkmans
The work described in this thesis was performed at the Department of Pathology (head Prof. dr. C.J.L.M. Meijer), VU University Medical Center, Amsterdam, The Netherlands. Financial support for this work was provided by ZonMw (Netherlands Organization for Health Research and Development; grant 30-05220).

Cover designed by Ridderprint Offsetdrukkerij BV, Ridderkerk
Printed by Ridderprint Offsetdrukkerij BV, Ridderkerk
Assisted by Jaap T. van Veldhuisen, medical photographer, Institute of Pathology, VU University Medical Center, Amsterdam

ISBN: 9789086591824
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Publication of this thesis was financially supported by TietoEnator, Werkgroep Cervix Uteri, Greiner, Thermo Fisher Scientific, Qiagen/Digene, and GSK.

PapilloCheck® HPV-Screening
Primary hrHPV testing in cervical screening: the arguments

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. L.M. Bouter,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der Geneeskunde
op dinsdag 15 januari 2008 om 10.45 uur
in de aula van de universiteit,
De Boelelaan 1105

doorn

Nicole Wilhelmina Johanna Bulkmans

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              dr. J. Berkhof
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Chapter 1

Introduction

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Chapter 1 Introduction

1. Cervical cancer

1.1 Epidemiology

Cancer of the cervix uteri is the second most common cancer among women worldwide, with an estimated 493,000 new cases and 274,000 deaths in 2002 (1). About 83% of the cases occur in developing countries, where cervical cancer accounts for 15% of female cancers and the cumulative risk to have cervical cancer before the age of 65 years is 1.5%. In developed countries, cervical cancer accounts for only 3.6% of new cancers and before the age of 65 the cumulative risk is 0.8% (1;2). In The Netherlands the age-standardised incidence rate (ASR) of cervical cancer was 6.2 per 100,000 person years in 2003 with an actual number of cervical cancers diagnosed of 584 (3). Cervical screening with call and recall systems as used in The Netherlands and several other countries, has contributed to lower age-standardised incidence rates (7.7 per 100,000 person years in Sweden during 1993-1997, 4.0 in Finland, 8.2 United Kingdom) compared to countries with opportunistic screening (10.8 Germany, 15.4 Poland,) or lacking any form of screening (35.9 Mali) (4).

Worldwide, the mortality to incidence ratio of cervical cancer is 55%. Survival rates vary again between regions with and without a well-organised screening programme, presumably because screening leads to earlier detection of cervical cancer and consequently to lower clinical stages with better survival (5-9). The five-years overall survival in The Netherlands is 71%, in the UK 68%, versus 30.5% in Harare in Zambia (2;3;10;11).

Based on epidemiological and biological data it has now been generally accepted that high-risk Human Papillomavirus (hrHPV) is the causative agent for cervical cancer being necessary for the development, maintenance and progression of precursor lesions to cervical cancer (12-15). Tobacco smoking and preceding Chlamydia trachomatis infection are hrHPV-independent cofactors that may modulate the risk of progression.
from an hrHPV infection to high-grade precursor lesions and cervical cancer (16;17).

1.2 Precursor lesions

The uterine cervix consists of an outer part of the cervix (ectocervix) and an inner part of the cervix (endocervix). The ectocervix and the vagina are covered with non-keratinising squamous epithelium and the endocervix and the endocervical canal are lined with glandular columnar epithelium. The border between the glandular and squamous epithelium is called the squamo-columnar junction (SCJ). From puberty onwards, the SCJ shifts outwards, i.e., towards the ectocervix, and at or round the menopause it shifts back inwards. In addition, shifting in- and outwards is influenced by hormonal levels, especially in pregnancy. The area across which the SCJ shifts is called the transformation zone. Partly, the transformation zone consists of metaplastic squamous epithelium. This metaplastic squamous epithelium is assumed to be more susceptible for HPV mediated transformation (15;18). As a result, most precursor lesions of cervical cancer develop from this metaplastic squamous epithelium.

Cervical lesions with the potential to progress towards invasive cervical carcinoma are histologically characterized by a disturbed epithelial architecture and cellular atypia and were originally classified as dysplasia. The concept of cervical intraepithelial neoplasia (CIN) was introduced in the late 1960’s, assuming that cervical cancer develops from these non-invasive pre-malignant stages (20). CIN lesions are classified into three groups according to the thickness of the epithelium involved in dysplastic changes. In CIN grade 1, less than one third of the epithelial layer is involved, in CIN grade 2 one to two thirds and in CIN grade 3 two thirds to full thickness of the epithelial layer show dysplastic features. CIN1 and CIN2 lesions are considered to be equivalent to mild and moderate dysplasia, respectively (21). CIN3 corresponds both with severe dysplasia and carcinoma in situ (CIS). Different grades of dysplasia may coexist at different sites within the same cervix. Alternatively, the Bethesda classification was introduced in the late eighties aiming at the distinction between lesions with a presumable low and high risk of progression to cervical cancer (22). In this grading system, which is not widely used in Europe, CIN1 is classified as low-grade squamous intraepithelial lesion (LSIL) while CIN2 and CIN3 are classified as high-grade SIL (HSIL). Squamous cell carcinoma is the most common histological type of cervical cancer, followed by adenosquamous carcinoma, adenocarcinoma, and, rarely, small-cell carcinoma.

1.3 Treatment of precursor lesions

Before the 1970s severe dysplasia and CIS lesions were treated by hysterectomy or conisation (a conus excision of the transformation zone), as it was impossible to evaluate the extent of the lesion. In the beginning of the 1970s usage of the
colposcope was advocated in the diagnosis of cervical abnormalities (23). The colposcopic impression is the anticipated severity of a cervical lesion as estimated from the magnified picture (at least 4 to 6 times) of the cervix uteri seen through the colposcope at the time of the optimal effect of application of a 3 to 5% acetic acid solution. The colposcopic impression is considered ‘satisfactory’ when the entire SCJ is visible and the upper (endocervical) limit of the cervical lesion can be seen. The classification is a prediction of the histopathological diagnosis and is related to the experience and skill of the colposcopist (24;25). Low- versus high-grade lesions show normal versus dense acteowhiteness, fine versus course mosaic or punctuation and fine versus thick leukoplakia, respectively. In high-grade lesions atypical vessel patterns can be present.

Colposcopically directed cervical biopsy specimens are taken for histopathological diagnosis, i.e. the gold standard to define the severity of the cervical lesion. According to the current Dutch guidelines, women with CIN1 are not treated and recalled for colposcopy after 1 year. Women with histologically CIN2 or CIN3 are treated to prevent the development of cervical cancer. Small and completely visible CIN2 lesions are by some gynaecologists not treated but guarded by a wait and see policy with a 6 months interval. The treatment of CIN lesions consists of removal of the transformation zone by LLETZ (large loop excision of the transformation zone), laser evaporation, cryocoagulation, cone biopsy, or hysterectomy depending on the severity of the lesion and the expertise of the attending gynaecologist. After treatment women are monitored for recurrence of the lesion by cytology testing at 6, 12 and 24 months and a colposcopy directed biopsy is indicated when an abnormal smear is found. After three consecutive normal smears women return to the cervical screening programme.

Caution is warranted in the treatment of young women since excisional procedures to treat CIN may lead to pregnancy-related morbidity such as preterm delivery, low birth weight, and premature rupture of the membranes (26). In general, LLETZ is preferred because it can be performed as an outpatient procedure and is followed by a histopathological assessment of the lesion removed.

2. Cervical screening

2.1 General aspects

Cervical cancer is considered to be a preventable disease, since the duration of the preclinical asymptomatic stage of precursor lesion to cervical cancer is estimated to be 12-25 years. Precursor lesions are detectable long before cervical cancer appears (27;28), and they can be treated by excision. For an effective population-based screening programme, the ten criteria as put forth by Wilson and Jungner in 1968 (29) should be fulfilled (Table 1).

<table>
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<th>Table 1: Principle and practice of screening for disease, Ten criteria by Wilson and Jungner</th>
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<td>1. the condition sought should be an important health problem</td>
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<td>2. there should be an accepted treatment for patients with recognised disease</td>
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<td>3. facilities for diagnosis and treatment should be available</td>
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<td>4. there should be a recognisable latent or early symptomatic stage</td>
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<td>5. there should be a suitable test or examination</td>
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<td>6. the test should be acceptable to the population</td>
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<td>7. the natural history of the condition, including development from latent to declared disease, should be adequately understood</td>
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<td>8. there should be an agreed policy on whom to treat as patients</td>
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<tr>
<td>9. the cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in ration to possible expenditure on medical care as a whole</td>
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<td>10. case finding should be a continuing process and not a “once and for all” project</td>
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Up to now cervical screening is based on cytology. Using cytology, abnormal cells of the (pre-) malignant stages may be detected, which has resulted in the organisation of population-based cervical screening programmes. It is currently estimated that systematic population-based screening with a call and recall system can reduce death rates from cervical cancer by 70% or more. A high participation rate and a reliable reproducible screening test are important parameters to achieve this reduction in mortality rate. For example in the United Kingdom, this reduction in mortality translates into at least 1000 lives per year saved among a population of 50 million (9;30;31).

To prevent abuse of screening, the Dutch government has passed the Dutch Population Screening Act (in Dutch WBO), in which the legal requirements for population-based screening are formulated. It includes the policy that effectiveness in reducing incidence and mortality has to be demonstrated by randomised trials and negative effects such as anxiety, false alarms, false reassurance, over diagnosis and over treatment have to be assessed. However, cervical screening had already been introduced before the screening act did pass, and the effect on incidence and mortality of cervical cancer has never been established in a randomised controlled trial. At the time of introduction, the positive effect of cervical screening seemed obvious because it was thought that cervical cancer precursor lesions could be detected easily and treated with an almost 100% cure rate. Indeed, studies analysing the effects of the introduction of mass screening have demonstrated that screening is effective in reducing incidence and mortality (7-9;30;32) as is shown in Figure 3.

2.2 Cytology as screening tool

The Pap test is based on cytomorphological examination of exfoliated cells from the transformation zone, squamous epithelial cells of the ectocervix and columnar cells from the endocervical epithelium (33). The Pap test is named after Papanicolaou who was the first to demonstrate that cervical cancer could be detected by this method (34). In the Netherlands the CISOE-A (in Dutch KOPAC-B) coding system is used (35-37). In this system, five items are scored and assigned values from 0 to 9: C for composition, I for inflammation, S for squamous epithelium, O for other abnormalities and endometrium and E for endocervical columnar epithelium. A stands for the adequacy of the smear. This leads to a five digit CISOE code that is converted into a Pap description. The CISOE code can also be translated into the revised Bethesda 2001 system (Table 3) (37). Only the SOE values influence the Pap class. Pap1 indicates normal cytology, Pap2: borderline dyskaryosis, Pap3a1: mild dyskaryosis, Pap3a2: moderate dyskaryosis, Pap3b: severe dyskaryosis, Pap4: suspected of carcinoma in situ, and Pap5: suspected of at least microinvasive cancer. In Table 3, the relationships between the different histologic classifications and their cytologic equivalents are presented.
Recently, Liquid-based cytology (LBC) has been introduced, under the impression that the sensitivity for ≥CIN2 lesions was higher. However a meta-analysis showed that the sensitivity of LBC for ≥CIN2 lesions was not increased compared to that of classical cytology (39). In contrast more smears with ASCUS and low-grade abnormalities were found resulting in an increase in follow-up of these women and thereby increasing costs. This was recently confirmed in a randomised controlled trial of LBC versus conventional cytology, showing no increase in sensitivity for the detection of histologically ≥CIN2 (40). The main advantage of LBC was an overall reduction of unsatisfactory smears. Yet, while other countries report a proportion of inadequate smears of 10% using conventional cytology, in The Netherlands the percentage of inadequate smears by conventional cytology in the screening programme is already very low, around 1%. Therefore, the advantage of a steep decrease of inadequate smears when using LBC does not count for the Netherlands. In contrast, an increase in inadequate smears has recently been reported (41). Convenience for the smear taker and the cytotechnician seems to be the only advantage when using LBC in the Netherlands at much higher costs compared to conventional cytology.

### 2.3 Current screening in The Netherlands

In the mid-seventies, population-based screening for cervical cancer was introduced in the Netherlands in three pilot regions, Nijmegen, Rotterdam and Utrecht, with the intention to compare cervical cancer rates in regions without screening. However, before drops in cancer rates could have been expected, screening programmes had been implemented in most parts of the Netherlands by the late seventies. In 1996, the
screening programme was revised and changed from a programme with a 3-year interval for women aged 35-53 years, into a 5-year interval for women aged 30-60 years, leaving the 7 invitations per lifetime unchanged. The main goal of this revision was to increase the effectiveness and to decrease the number of smears taken outside the cervical screening programme.

For the most recent schedule, it is estimated that by responding to all 7 invitations the risk of dying from cervical cancer is reduced by 75% (42). Assuming 75% attendance, the mortality reduction for the new programme is predicted at approximately 50% (42). In the Netherlands the attendance rate (defined as the number of women who actually undergo screening after receiving the invitation) is 66% and the coverage (defined as the proportion of women who had a smear taken in the preceding 5-years) is 73% (43;44) (43;45) (46). The attendance rate was the lowest (i.e., 52%) among the younger women (aged 30 years) (43;47). The higher coverage rate was attained because of opportunistic screening outside the screening programme. Hence the proportion of women protected by mass screening was higher than suggested by the programme attendance rate. Since it is known that 50% of the women with invasive cervical cancer arise in women who are not adequately screened, an increase of the participation is desirable (32;48) (9;48-50).

In the Netherlands, cervical smears are taken by the general practitioner or assistant. Women with normal cytology are recalled at the subsequent screening round after 5 years. Women with moderate dyskaryosis or worse (>BMD; Pap2/3a1) are advised to repeat the tests after 6 and 18 months. Women who still have BMD or worse after either 6 or 18 months are referred to colposcopy. Women with regression to normal cytology after 18 months are not recalled until the subsequent screening round.

Results of the in 1996 revised screening programme were reported for the year 2001 (43). In 2001, 823,000 smears were taken, of which 697,000 were primary screening smears and 126,000 considered follow-up smears after a previous abnormal result. Of the screening smears 96.3% had a negative result (Pap 1) and these women were recalled after 5 years, 2.1% were Pap2/3a1 (borderline or mild dyskaryosis, BMD) and were repeated at 6 and 18 months, and 0.6% were Pap3a2 (moderate dyskaryosis or worse, >BMD) and these women were directly referred for colposcopy. CIN was diagnosed in 8,000 women. The incidence rate for a CIN lesion was 1.4 per 1000 women at risk, i.e., 0.5 CIN1, 0.3 CIN2, and 0.6 CIN3 per 1000 women at risk (43). Since the introduction of the new guidelines in 1996, the number of screening smears diagnosed as Pap2 (borderline dyskaryosis) has decreased from 10% to 2%. As a result, the number of women who require follow-up smears reduced sharply. However, the number of ≥CIN2 lesion detected and the proportion of women referred to a gynaecologist remained the same (43;51).

The introduction of population-based cervical screening programmes in developed countries has led to a drastically reduced incidence of cervical cancer (9;30;32;48;52;53), including in The Netherlands (Figure 4).
However, cervical screening by cytology has shortcomings. The sensitivity of cytology for the detection of high-grade precursor lesions or cervical cancer is only about 65% (54-56). Since cytology has limited sensitivity for the detection of pre-cancerous lesions and treatable cancers, repeated cytology over short intervals is necessary to achieve program efficacy. Only repeatedly normal cytology denotes safety. In addition, the decrease in incidence of cervical carcinoma is contributable to a decrease in incidence of squamous cell carcinoma. There was no decrease in the incidence of adenocarcinoma (Table 4). Consequently, there is a need for a better screening test.

Table 4: Incidence of cervical cancer in The Netherlands 1989-2003 (ESR European Standardised Rate) (57)

<table>
<thead>
<tr>
<th>Year</th>
<th>SCC</th>
<th>AdCa</th>
<th>Total</th>
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<tr>
<td>89</td>
<td>7.1</td>
<td>1.4</td>
<td>9.1</td>
</tr>
<tr>
<td>90</td>
<td>7.0</td>
<td>1.8</td>
<td>8.8</td>
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<tr>
<td>91</td>
<td>6.4</td>
<td>1.7</td>
<td>8.1</td>
</tr>
<tr>
<td>92</td>
<td>6.8</td>
<td>1.6</td>
<td>8.4</td>
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<tr>
<td>93</td>
<td>6.3</td>
<td>1.7</td>
<td>8.0</td>
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<tr>
<td>94</td>
<td>6.2</td>
<td>1.6</td>
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<td>6.1</td>
<td>1.6</td>
<td>8.0</td>
</tr>
<tr>
<td>97</td>
<td>6.1</td>
<td>1.8</td>
<td>8.3</td>
</tr>
<tr>
<td>98</td>
<td>6.1</td>
<td>1.7</td>
<td>8.2</td>
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SCC, squamous cell carcinoma; AdCa, adenocarcinoma; ESR, European Standardised Rate

The costs of the screening programme are € 26 million yearly, and are financed by the AWBZ (exceptional medical costs act). Yearly € 9 million is spend on regional organisation and administration, including invitations, information, and allowance for self-inviting general practitioners. € 16 million is intended for making and reading of the cervical smears. One million is intended for monitoring, evaluating and quality improvement of the screening programme (58). Attending the cervical screening programme has no costs for the women. The costs of a cervical smear, including costs for organisation and invitation, are € 48,-. In case of an abnormal smear result, the woman has to have the smear repeated or she is referred to a gynaecologist. Both the costs of repeated smears, referral, treatment and monitoring after treatment are financed by the health insurance companies. For the current screening programme with 7 smears per women per lifetime, estimated costs are € 12.500 per year of life saved. The upper-limit for a screening programme to be cost-effective in the Netherlands is set at € 20.000 per year of life saved. Therefore the screening programme for cervical cancer is considered cost-effective (44). Compared to screening for breast cancer (€ 3.500 per years of life saved), the cost-effectiveness for
cervical screening is less favourable. This is one of the reasons why the number of smears per women per lifetime is not increased, and a more cost-effective screening strategy with an other test would be plausible.

3. Human Papillomavirus (HPV)

3.1 Epidemiology

Genital HPV is sexually transmitted and the majority of women are tested positive for HPV after starting sexual activity. The cumulative incidence of HPV infection among women aged 15-19 in England was 44% over a 3-year period and increased to 60% at 5 years (59). Genital HPV infections are very common, though most infected individuals (80%) clear the virus without ever developing clinically recognized manifestations. In a prospective study of female college students, approximately 70% of women no longer had detectable levels of HPV DNA within 12 months of follow-up after incident HPV infection. Thus, few HPV-infected individuals (1%) ultimately show progression to invasive cervical cancer. Detection of HPV varies greatly by age and by geography. The prevalence of HPV infection is highest among young women and appears to drop off with increasing age (60). In Europe and North America, the age-specific prevalence curve of cervical HPV infection shows a peak at 18-25 years and then declines sharply to 2-5%. HPV infection also appears to be very common in men, though it has not been studied as extensively as infection in women. Using PCR analysis of genital samples, the prevalence of HPV in a Dutch male non-STD hospital population was 25% (61). The prevalence varies about 20-fold between different regions, from 1.4% (95%CI 0.5-2.2) in Spain to 25.6% (95%CI 22.4-28.8) in Nigeria (62).

3.2 HPV genome and viral life cycle in transient infections

Human papillomavirus particles consist of ~8000 base-pair long circular DNA molecules wrapped into a protein shell that is composed of two molecules (L1 and L2) and form an icosahedral capsid (Figure 5). L1 and L2 play an important role in mediating efficient virus infectivity. The genome has the coding capacity for these two late proteins and at least six so-called early proteins (E1, E2, E4-7) that are necessary for the replication of the viral DNA and for the assembly of newly produced virus particles within the infected cells (Figure 6; Table 5) (16).

![Figure 5](image-url)
Figure 6: Schematic presentation of the HPV genome showing the arrangement of the early E or non-structural genes, the capsid genes (L1 and L2) and the upstream regulatory region (URR) (16).

| Table 5: Functions of the early E and Late L genes of HPV |
|-----------------|----------------------------------------------------------|
| E1              | basal DNA replication                                     |
| E2              | basal DNA replication; acts as a transcription factor and can regulate the viral early promotor; by down-regulating the expression of E6 and E7, and thereby controlling expression of the viral oncoproteins E6 and E7 |
| E4              | maturation of the virus; induces the collapse of the cytoplasmic cytokeratin network suggesting a role in the release of viral particles |
| E5              | affects the recycling of growth factor receptors on the cell surface |
| E6              | targets p53 for proteolytic degradation                   |
| E7              | binding of E7 to pRB activates the E2F transcription factor, which triggers the expression of proteins necessary for DNA replication |
| L1              | capsid gene                                              |
| L2              | capsid gene; facilitates the transfer of viral DNA to the nucleus |
| URR             | regulation of gene expression, replication of the genome, and it’s pack-aging into virus particles |

Infection by papillomaviruses requires that viral particles gain access to the epithelial basal layer and enter the dividing basal cells (64). The replication cycle within the epithelium can be divided into two parts. First, the viral genome is replicated and established as stable episomes in cells of the basal layer. They are maintained for varying periods of time at a low copy number (10-200) within the initially infected but still replicating cells, where most viral proteins including the E6 and E7 oncoproteins are expressed at low levels. In fact, expression of these proteins is under the influence of factors determining cellular differentiation. Once the basal cells are pushed to the suprabasal compartment, cells normally exit the cell cycle and begin the process of terminal differentiation in order to produce the protective barrier that is normally provided by the epithelium. Papillomaviruses replicate in this suprabasal compartment where expression of viral genes is elevated. For their release into the environment, the virus takes advantage of the disintegration of the epithelial cells that occurs as a consequence of their natural turnover at the superficial layers (productive infection). Primary HPV infections targeting differentiated, more superficial cells are a priori transient, since the viral DNA will be lost as the infected cells are shed during the terminal differentiation process.

3.3 HPV-mediated cervical carcinogenesis in transforming infections

Both the genetic background of the host with regard to immune surveillance mechanisms and the nature of the infected target cells are decisive for the development of CIN lesions. It is assumed that it takes on average 12-15 years before a persistent hrHPV infection may ultimately lead to cervical carcinoma (28,65). It has been estimated that approx. 20% of the productive HPV infections will develop morphological changes read as CIN1 to CIN2 lesions by pathologists. These lesions are considered as the cytopathologic effect of a productive HPV infection and often regress. When the virus integrates into the cellular genome, deregulation of the E6 and E7 genes in the proliferating basal cells results in a transforming infection. In that case, the expression of E6 and E7 becomes independent of cellular differentiation. By
Figure 7: Progression model of cervical cancer based on in vitro transformation steps and data from clinical samples. TSGs = tumour suppressor genes. ↑ indicates increased activity resulting from (epi)genetic alteration(s) ↓ indicates decreased activity resulting from (epi)genetic alteration(s), such as deletion or promoter hypermethylation (66).

pathologists, the resulting lesions are read as CIN2 to CIN3, thus showing that CIN2 is a heterogeneous group of lesions consisting partly of mainly regressing productive infections and partly persistent transforming lesions. For progression of CIN lesions HPV persistence is necessary. Approximately 40% of CIN3 lesions can progress to cancer (9). Many of the clinically relevant CIN 2/3 lesions may be rapidly induced within 3-5 years following infection (67). When HPV persist it takes on average another 10–12 years to develop invasive cervical cancer (28;65).

The critical molecules in the process of HPV-mediated transformation are the viral proteins E6 and E7, which interact with a number of cellular proteins. The best-characterized interactions are those with the proteins pRB and p53, which are central molecules in cell cycle control, and remarkably, are mutated in many human cancers. Binding of E7 to pRB activates the E2F transcription factor, which triggers the expression of proteins necessary for DNA replication (68). Unscheduled S-phase entry would normally lead to apoptosis by the action of p53; however, in HPV-infected cells, this process is counteracted by the viral E6 protein, which targets p53 for proteolytic degradation (69). As a consequence, the cell cycle control is abolished and normal keratinocyte differentiation is retarded (64). In transforming infections, constant over activity of the viral proteins E6 and E7 in proliferating cells leads to genomic instability, accumulation of gene mutations, loss of cell-growth control, and ultimately may give rise to cancer (Figure 7) (70). During tumour progression, the viral genomes often integrates into the host chromosome, which results in a constant level of E6/E7 proteins via stabilization of the mRNA, by the influence of modified chromatin structures or by loss of negative regulation of transcription mediated by the viral E2 protein (71).

3.4 HPV types

Currently over 100 different HPV types have been identified based on DNA sequence analyses (72;73). Other papillomavirus types have also been identified in mammals and birds. At the evolutionary level, HPVs fall into a number of
distinct groups or genera, and the lesions they cause have different characteristics.

An HPV genome is now defined as a new HPV type when it shows more than 10% difference in nucleotide sequences of the E6, E7, and L1 genes compared to those of any previously known type. HPV subtypes are defined as having a sequence difference between 8 and 10% and HPV intratype variants are defined as having less than 2% nucleotide sequence difference with any given HPV type (74).

Figure 8: HPVs and their association with cervical disease

HPVs are contained within several evolutionary groups. HPV types that infect the cervix come from the Alpha group which contains over 60 members. HPV types from the Beta, Gamma, Mu and Nu groups primarily infect cutaneous sites, e.g., HPV2 which cause common warts (75).

The largest group of HPVs comprises the Alpha papillomaviruses. More than 30 of these Alpha HPV types are known to infect cervical epithelium. These types can be divided into low-risk HPV types, associated with genital warts and non-progressing low-grade CIN lesions, and high-risk types associated with high-grade CIN lesions and cervical cancer.

Figure 9: High-risk types of HPV are indicated with orange boxes, and low-risk types of HPV are indicated with blue boxes (75).

It was shown that only the E6 and E7 genes of high-risk types were able to immortalize human epithelial cells in tissue culture and fulfil the oncogenic changes resulting in the progression to cervical cancer. HPV16 is the most prevalent high-risk HPV type in the general population, and is responsible for approximately 50% of all cervical cancers. Based on pooled analyses of case-control studies involving large series of women with cervical cancer, performed by the International Agency for Research on Cancer (IARC), an epidemiological classification could be made in which 15 types were assigned as high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82), 3 types as probably high-risk (26, 53, and 66) and 12 as low-risk (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and cand89) (76). A good agreement exists between the phylogenetic and the epidemiological classification of HPVs into high-and low-risk. There is an impression that the diverse high-risk HPV types confer a difference in risk for the development of ≥CIN2.

3.5 HPV detection methods

The currently most used widely applied HPV-DNA detection methods are based on two principles. The first, as used in the Hybrid Capture
2 (HC2) assay, involves hybridisation of HPV target DNA with a cocktail of full-length HPV type-specific RNAs, followed by capture of DNA/RNA hybrids on a solid phase. Subsequently, signal amplification is achieved by the binding of multiple conjugated antibodies that specifically recognize DNA/RNA hybrids. The HC2 system has been automated (Rapid Capture System) which enables high throughput mass screening.

The second principle is based on PCR amplification of HPV target DNA by so-called consensus or general primers that bind to highly conserved regions within the L1 open reading frame of all genital HPV genotypes. In case of consensus primers conditions can be chosen to accept a small number of mismatches between the primers and target sequences to ensure amplification of all relevant HPV types. Alternatively, primers with inosine residues at ambiguous base positions can be used or mismatches can be avoided by using multiple overlapping primers that perfectly match the target sequences of various HPVs. The latter results in a so-called multiplex PCR. The most widely used consensus PCR methods are based either on the MY09/11 (or PGMY, a multiplex assay redesigned thereof) and GP5+/6+ primer sets (77;78), both of which amplify a region of the L1 gene for genital HPV types. The MY09/11 (PGMY) primers produce a PCR product of 450 base pairs, whereas the GP5+/6+ primers produce an amplimere of about 150 base pairs, which makes the latter less sensitive for DNA cross linking by formalin fixation. The SPF10 PCR primer set amplifies a fragment of only 65 base pairs within the L1 region. Several read-out systems have been described for consensus PCR assays, but enzyme immuno-assays (e.g., EIA, (79;80) using type-specific oligoprobes (either individually, or in a cocktail, or reverse line blot assays (e.g., LiPA (81), or RLB (82)) are still the most commonly used.

The differences in HPV detection rates of these tests can be attributed to differences in test-characteristics, such as a.o., direct hybridisation versus target amplification, size of the amplimere, and multiplex or degenerate primers versus conditions of mismatch acceptance. In addition, the number of HPV types detected can be different. However, all these methods have the ability to detect all relevant HPV types, simply by adapting the composition of the probes. The HC2 method has a tendency to show a certain degree of cross-reactivity with HPV types not represented in the probe mixture (83). The analytical sensitivity of the HC2 assay is at the picogram level of HPV DNA, whereas that of the PCR assays is at the sub-picogram level. Some assays are routinely performed on crude cell extracts of cervical scrapes (79), whereas others make use of isolated DNA, which results in an increased sensitivity (80;84).

The test with the best analytical sensitivity, i.e., the proportion of HPV-positive women who are correctly identified by a given test, is the better one to estimate the epidemiological burden of HPV infections, necessary for setting up or monitoring HPV vaccination studies. The clinical sensitivity identifies the proportion of women with disease who are correctly identified by a positive HPV test. Although most tests have a similar clinical sensitivity, the clinical specificity (the proportion of women without ≥CIN2 correctly identified by a negative test result) differ markedly (85). A high clinical specificity makes a better distinction between so-called clinically relevant and irrelevant HPV infections, which distinction should be made when considering HPV tests for primary screening, triage policies, or post-treatment monitoring. There is no need to detect women with a transient HPV
infection, since they will not develop ≥CIN2. When combining the data from various studies, it can be concluded that SPF10 has the highest analytical sensitivity, whereas HC2 and GP5+/6+-PCR have the highest clinical specificities (85). The SPF assay detected significantly more HPV-DNA in cervical scrapes of women with normal cytology than the GP5+/6+-PCR assay, whereas no significant differences in estimated HPV prevalence rates were found in women with ≥CIN2 lesions (85). The GP5+/6+-PCR and HC2 methods apparently detect less transient HPV infections in women with normal cytology compared with the SPF10 method, but still have an excellent negative predictive value for ≥CIN2 in women with both normal and abnormal cervical scrapes (86).

3.6 HPV testing as screening tool

Since hrHPV is the causative agent for developing cervical cancer (12-14), hrHPV testing might be an effective primary screening tool. The question whether hrHPV testing has additional value to cytology based screening for cervical cancer still has to be answered. In addition hrHPV testing could also be effective for triage and post-treatment strategies.

Cross-sectional studies have demonstrated that combined testing by cytology and hrHPV has a higher sensitivity and negative predictive value (NPV) at the cost of a slightly lower specificity than cytology alone for the detection of prevalent high-grade cervical intraepithelial neoplasia and cervical cancer (≥CIN2) (87-90). A recent overview of European and North American studies identified the sensitivity of hrHPV testing for the detection of ≥CIN2 at 96% (range: 85-100%) (55). Yet, cross-sectional studies do not permit long-term evaluation of different screening strategies on the detection of ≥CIN2, since all women with abnormal results from either test underwent short-term follow-up by colposcopy and treatment. It cannot be excluded that a considerable number of the lesions that are additionally detected by hrHPV testing might regress spontaneously and therefore would not contribute to cervical cancer development. Large-scale randomised controlled trials with long-term follow-up up to and including the next screening round will provide a higher standard of unbiased evaluation of different screening strategies. These data can be used to decide whether and how to use hrHPV testing in cervical screening programmes.

Currently, randomised controlled trials in Sweden, Italy, United Kingdom, Finland, Canada, and the Netherlands are being conducted to establish the performance of HPV testing as a primary cervical screening test (38;40;91-95).

4. Outline of this thesis

In The Netherlands three important studies considering hrHPV testing in population-based screening have been initiated by the Pathology department of the VU University Medical Center. The Amstelveen cohort study, including 2810 women is the first prospective study with 5-years follow-up in the setting of a population-based screening programme. The POBASCAM trial, including 44,102 women, is a large randomised implementation trial embedded in the regular population-based screening programme including two consecutive screening rounds, using the GP5+/6+ PCR EIA test. The VUSA-screen is a screening trial of 50,000 women embedded in regular population-based screening, comparing the use of the HC2 test combined with cytology versus cytology alone. Results of the Amstelveen cohort study and the POBASCAM trial are presented in this thesis.
The Population Based Screening Study Amsterdam (POBASCAM) has been initiated to evaluate the effectiveness of hrHPV testing by GP5+/6+ PCR-EIA in conjunction with cytology (Intervention Group) to that of classical cytology (Control Group). In this thesis we will describe baseline results, participation rates, risks and type-specific clearance rates, ≥CIN2 detection rates and referral rates of the POBASCAM trial.

The outcome measure of the trial is the proportion of histologically confirmed ≥CIN3 lesions in each study arm up to and including the next screening round after 5 years. The data obtained will be used in modelling studies, including a cost-effectiveness study, to advise the Dutch Ministry of Public Health in deciding whether cervical screening should be based on hrHPV testing or combined hrHPV and cytology testing instead of cytology alone.

Since infection with high-risk human papillomavirus (hrHPV) is considered to be the cause of cervical carcinoma, it has been suggested that adding hrHPV testing to cervical screening might improve screening in terms of reducing false-positive and false-negative smears. However, long-term data are needed before hrHPV testing can be implemented in population-based cervical screening. Therefore, in Chapter 2, based on the data derived from the Amstelveen cohort study, we answer the question: What are the prospective long-term (5 years) predictive values of routine hrHPV testing in population-based cervical screening in The Netherlands?

To evaluate prospectively in a large-scale population-based primary screening setting whether adding hrHPV testing to classical cytology might result in more efficient screening, we initiated the POBASCAM trial. In Chapter 3 we present the trial design of POBASCAM and the cross-sectional baseline data of the 44,102 enrolled women.

hrHPV is a sexually transmitted agent and its perceived association with sexually transmitted diseases may hamper the introduction of hrHPV testing in cervical screening programmes. In Chapter 4 we answer the question: Does implementation of hrHPV in cervical screening result in a decreased participation rate?

In order to get insight into the relationship between hrHPV type, cervical cytological abnormalities and underlying CIN lesions, we analysed by cross-sectional analysis the intake data of the POBASCAM study. In Chapter 5 we answer the question: Do women with normal cytology have a different type-distribution of the 14 hrHPV types compared to women with BMD and >BMD with underlying ≥CIN2?

The inclusion of hrHPV testing in cervical screening requires efficient management, as many hrHPV infections are transient. Therefore data about risk for ≥CIN2 and clearance time for different HPV genotypes are needed. We investigated the potential value of hrHPV genotyping for risk management in women with normal and BMD smears. In Chapter 6 we answer the question: What are the hrHPV type-specific 18-month risks of high-grade CIN in women with normal cytology and in women with BMD participating in a population-based screening programme?

The positive predictive value of a single positive hrHPV test remains low for women with a normal smear or mild cytological abnormalities, and referral rates for colposcopy may increase substantially with combined testing. Hence, implementation of hrHPV testing needs to be preceded by an evaluation of various screening strategies using hrHPV and cytological testing. One
of them is evaluation of the combined test results after 6 months. In Chapter 7 we answer the question: What is the risk of ≥CIN2 based on cytology and hrHPV testing at baseline and at six months?

Besides the risk for ≥CIN2 posed by different HPV types it is important to have clearance data for women with different HPV genotypes. In the search of an optimal screening algorithm using hrHPV testing, it is essential to determine the time point at which the majority of the screening participants has cleared the virus and can be referred back to regular screening, and whether different hrHPV types show difference in clearance rates. In Chapter 8 we answer the question: What are the hrHPV type-specific clearance rates after 6 and 18 months for women with normal cytology and for women with BMD?

Only two assays that detect DNA of hrHPV types as a pool have proven to be of clinical value in longitudinal studies involving large cohorts of women, i.e., HC2 and GP5+/6+ PCR). In Chapter 9 we answer the question: Is there a difference in clinical performance of the automated HC2 assay and the consensus GP5+/6+ PCR method for the detection of ≥CIN3 in a population-based cervical screening programme?

Although it is known that HPV testing has a higher sensitivity for the detection of ≥CIN3 than cytology, it has been questioned whether these lesions are clinically relevant, i.e., non-regressing. In Chapter 10, based on data derived from the POASCAM trial with five years of follow-up we answer the following questions: Does the long-term effectiveness of cervical screening improve when HPV DNA testing is implemented? Are the additionally detected ≥CIN2 lesions by hrHPV testing clinically relevant, i.e., non-regressing? What are the five-year cumulative ≥CIN2 risks after normal cytology and after a negative hrHPV test result?

Finally in Chapter 11 we discuss our findings as presented in the preceding chapters in relation to possible implementation of hrHPV testing in primary cervical screening.
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Chapter 1 Introduction


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Chapter 2

Long-term protective effect of high-risk Human Papillomavirus testing in population-based cervical screening

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*British Journal of Cancer* 2005; 92(9): 1800-1802
Long-term protective effect of high-risk human papillomavirus testing in population-based cervical screening

Cervical screening by cytology is known to yield a substantial proportion of both false-positive and false-negative smears. Since infection with high-risk human papillomavirus (hrHPV) is considered to be the cause of cervical carcinoma, it has been suggested that adding hrHPV testing to cervical screening might improve screening in terms of reducing false-positive and false-negative smears (Rozendaal et al., 2000; Cuzick et al., 2003). However, long-term data are needed before hrHPV testing can be implemented in population-based cervical screening. Here, we present for the first time the prospective long-term (5 years) predictive values of routine hrHPV testing in population-based cervical screening in The Netherlands.

MATERIAL AND METHODS

A cohort of 3170 women (mean age 45 years; range 29–61 years) was enrolled from March 1995 till October 1998 by 60 general practitioners in the small district of Amstelveen, The Netherlands. In all, 194 women were excluded because of abnormal cervical cytology and/or histology during 2 years preceding the intake, 159 women because of a negative β-globin PCR test, and seven women because of inadequate cytology, leaving 2810 women for analysis. Cytological screening was performed according to the CISOE-A classification, routinely used in cervical screening in The Netherlands, and the referral policy was according to the nationwide guidelines (Bulk et al., 2004). High-risk human papillomavirus testing was performed by GPS + β + PCR-EIA, using a cocktail probe of 14 hrHPV types, and independent of cytology results. The hrHPV test result was blinded. Informed consent was obtained and the study was approved by the Medical Ethics Committee of the VU University Medical Center. The primary end point of the study was the detection of histologically proven cervical intraepithelial neoplasia grade 3 or cervical carcinoma (≥ CIN3) up to and including the next screening round (after 5 years). Follow-up data were retrieved from the Dutch nationwide pathology registry (PALGA) in 2004.

Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of cytology, cytology and hrHPV testing combined, and hrHPV testing, for the detection of prevalent and incident lesions ≥ CIN3 were computed using two by two tables. Results are presented as percentages with 95% confidence intervals (95% CI). To determine whether any gain in test performance from the addition of the second test (hrHPV testing) was greater than that expected if the second test offered no diagnostic information, expected performance characteristics for cytology combined with a random test having the similar diagnostic information, expected performance characteristics for cytology testing combined, and hrHPV testing, the for the detection of prevalent and incident lesions ≥ CIN3 were computed using two by two tables. Results are presented as percentages with 95% confidence intervals (95% CI). To determine whether any gain in test performance from the addition of the second test (hrHPV testing) was greater than that expected if the second test offered no diagnostic information, expected performance characteristics for cytology combined with a random test having the similar prevalence as hrHPV were computed (Franco and Ferenczy, 1999).

RESULTS

Of 2810 women for analysis at baseline, 2687 (95.6%) women had normal cytology, of whom 77 (2.9%) had a positive hrHPV test. Among 111 (4.0%) women with borderline or mild dyskaryosis (BMD), 16 (14.4%) women were hrHPV positive, as were 11 (91.7%) of 12 (0.4%) women with moderate dyskaryosis or worse (≥ BMD). Among the 123 (5.2%) women who had abnormal cytology, nine (7.3%) cases of prevalent lesions ≥ CIN3 were present (including three squamous cell carcinomas) (Table 1), resulting in a detection rate of 0.3% lesions ≥ CIN3 by cytology among all 2810 women at baseline. All women with lesions ≥ CIN3 at baseline had a positive hrHPV test.
Human papillomavirus testing in cervical screening
NWJ Bulkmans et al

Table 1 Baseline histology stratified to cytological diagnosis and hrHPV status

<table>
<thead>
<tr>
<th>Baseline cytology</th>
<th>Baseline hrHPV</th>
<th>ScC n (%)</th>
<th>Aden. Ca n (%)</th>
<th>CIN3 n (%)</th>
<th>CIN2 n (%)</th>
<th>Lesser abnormality n (%)</th>
<th>Normal n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>77 (100)</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>77 (100)</td>
</tr>
<tr>
<td>BMD</td>
<td>+</td>
<td>3 (18.8)</td>
<td>3 (18.8)</td>
<td>—</td>
<td>—</td>
<td>1 (1.1)</td>
<td>2 (12.5)</td>
<td>8 (50.0)</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>3 (18.8)</td>
<td>2 (1.2)</td>
<td>—</td>
<td>3 (18.8)</td>
<td>—</td>
<td>6 (3.6)</td>
<td>88 (92.6)</td>
</tr>
<tr>
<td>&gt;BMD</td>
<td>+</td>
<td>3 (27.3)</td>
<td>2 (18.2)</td>
<td>—</td>
<td>3 (27.3)</td>
<td>—</td>
<td>1 (10.0)</td>
<td>11 (100)</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>3 (27.3)</td>
<td>3 (2.7)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>—</td>
<td>3 (0.1)</td>
<td>6 (0.2)</td>
<td>—</td>
<td>6 (0.2)</td>
<td>11 (0.4)</td>
<td>2784 (99.1)</td>
<td>2810 (100)</td>
</tr>
</tbody>
</table>

Scc, squamous cell carcinoma; Aden. Ca, adenocarcinoma; CIN1–3, cervical intra-epithelial neoplasia grade 1–3; lesser abnormality, CIN1 or abnormal smear; normal, histological or cytological normal diagnoses; BMD, borderline or mild dyskaryosis (Pap 2–3a mild dyskaryosis); >BMD, moderate dyskaryosis or worse (Pap 3a moderate dyskaryosis or worse); FU, follow-up. Baseline histology is presented for women with BMD and >BMD, with the annotation that for women with BMD histology was obtained after a serial abnormal smear after 6 or 18 months. For women with normal cytology (Pap 1), the follow-up data are presented in Table 2.

Table 2 Years of follow-up of women with normal cytology stratified to final histological diagnosis

<table>
<thead>
<tr>
<th>Baseline status</th>
<th>FU</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 year</td>
</tr>
<tr>
<td>Normal cytology, hrHPV positive</td>
<td></td>
</tr>
<tr>
<td>≥CIN3</td>
<td>1</td>
</tr>
<tr>
<td>CIN2</td>
<td>—</td>
</tr>
<tr>
<td>Lesser abnormality</td>
<td>—</td>
</tr>
<tr>
<td>Normal</td>
<td>4</td>
</tr>
<tr>
<td>Normal cytology, hrHPV negative</td>
<td></td>
</tr>
<tr>
<td>≥CIN3</td>
<td>1</td>
</tr>
<tr>
<td>CIN2</td>
<td>—</td>
</tr>
<tr>
<td>Lesser abnormality</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
</tr>
</tbody>
</table>

Of the total number of 2687 women with normal cytology, 450 did not have follow-up data: 146 women of 59–61 years of age were not expected to have another cervical screening and 304 did not have another screening because of other reasons. Normal cytology, hrHPV positive: ≥CIN3 = CIN2 or CIN3; CIN2, CIN3 or lesser abnormality; Normal, histological or cytological normal diagnoses. *One woman was diagnosed with squamous cell carcinoma, the other with CIN3, adenocarcinoma.

Table 3 Observed performance characteristics for lesions ≥CIN3 for cytology, cytology+hrHPV and hrHPV testing

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytology</td>
<td>64.3 (35.6–86.0)</td>
<td>95.1 (94.2–96.0)</td>
<td>7.3 (3.9–13.3)</td>
</tr>
<tr>
<td>Cytology+hrHPV</td>
<td>92.9 (64.2–99.6)**</td>
<td>92.7 (91.5–93.7)*</td>
<td>7.0 (4.2–11.7)†</td>
</tr>
<tr>
<td>hrHPV</td>
<td>92.9 (64.2–99.6)**</td>
<td>96.7 (95.9–97.4)*</td>
<td>14.6 (8.7–23.4)**</td>
</tr>
</tbody>
</table>

Expected performance characteristics for cytology and a combined test, which assumes that the second test is random with the same prevalence as hr-HPV (3.7%), with respect to disease detection: sensitivity 65.6 (36.8–86.9), specificity 91.6 (90.3–92.6), PPV 4.4 (2.4–8.2), NPV 99.78 (99.47–99.91); P-values for differences in performance characteristics compared to cytology: *P<0.05; **P<0.1; †P>0.1.

The median follow-up time until histological or cytological diagnoses was 4.6 years (range 0.1–8 years). The follow-up results of women with normal cytology at baseline are presented in Table 2. Of the 2687 women with normal cytology, 304 did not have follow-up data registered in PALGA. Another 146 women were excluded because they reached age 60 years or over and therefore were not called for further screening, leaving 2237 (83.3%) women with normal cytology for follow-up analysis. Among the 62 women of this cohort with an hrHPV-positive test at baseline, four (6.5%) cases had incident lesions ≥CIN3. Among the 2175 women with a negative hrHPV test, one (0.05%) lesion ≥CIN3 (an adenocarcinoma) was registered within this follow-up period. This woman with a symptomatic adenocarcinoma, who was tested hrHPV negative by GP5+ and GP6+ primers, for hrHPV-positive women with normal cytology, the relative risk to develop lesions ≥CIN3 in 5 years was 140.3 (95% CI 15.9–1237.3) compared to hrHPV-negative women with normal cytology. The overall detection rate after 5 years in this study of lesions ≥CIN3 by cytology was 0.5%.

The performance characteristics of cytology, cytology and hrHPV combined, and hrHPV testing, for the detection of lesions ≥CIN3 are given in Table 3. Combined screening revealed a much higher sensitivity, at the cost of a small decrease of specificity, and a higher NPV at the next screening interval (after 5 years) than cytology alone. Combined testing was more sensitive than cytology alone (92.9 vs 64.3%, respectively; P = 0.065), but was less specific (92.7 vs 95.14%, respectively; P < 0.001), and had an increased NPV (99.95 vs 99.78%, respectively, P = 0.109) with a similar PPV (7.0 vs 7.3%; P = 0.922). High-risk human papillomavirus testing alone
was more sensitive than cytology alone (92.9 vs 64.3%, respectively; \( P = 0.065 \)), more specific (96.8 vs 95.1%, respectively; \( P = 0.005 \)), and had an increased NPV (99.6 vs 99.78%, respectively; \( P = 0.098 \)) and an increased PPV (14.6 vs 7.3%, respectively; \( P = 0.085 \)). Comparing the sensitivity and NPV of cytology and hrHPV combined screening to cytology with a random test, no different significance levels were revealed as when compared to cytology alone.

**DISCUSSION**

Combined screening revealed a much higher sensitivity, at the cost of a small decrease of specificity, and a higher NPV at the next screening interval (after 5 years) than cytology alone. Cytology alone had reasonable performance characteristics, but on adding hrHPV testing we can achieve much better performance characteristics in population-based screening.

The overall detection rate by cytology of 0.3% lesions \( \geq \text{CIN}3 \) at baseline and 0.5% after 5 years in this study is comparable to the detection rate of lesions \( \geq \text{CIN}3 \) in cervical cancer screening in The Netherlands (Bos et al., 2002; Anttila et al., 2004). The high NPV of the combination of a negative hrHPV test and a normal smear is in accordance with Clavel et al. (2004), who reported an NPV of 99.9% in a partly hospital-based population, with a much shorter interval (median 2.8 years) for women of 15–79 years of age. Sherman reported an NPV of 99.2% for hrHPV in cervical lavage specimens during annual screening for women of 16–94 years of age (Sherman et al., 2003). The data are also in line with the data of the Manchester cohort (Peto et al., 2004).

Our data are the first that were prospectively obtained in population-based screening, with a 5-years screening interval in women 30–60 years of age. Some methodological aspects of this study need to be discussed. The fact that histology was not obtained in all women might induce a verification bias in advantage of combined screening and hrHPV testing. However, women were followed according to current practice standards in nationwide screening with cytology after 5 years, and in this setting women with normal cytology are considered to be free of disease. As indicated by the overlapping 95% CIs, the gains in sensitivity and NPV by combined testing or hrHPV testing compared to cytology alone were not significant (\( P < 0.05 \)). This might be due to our relatively small population with a low prevalence of lesions \( \geq \text{CIN}3 \), resulting in wide 95% CIs. However, with this low prevalence of lesions \( \geq \text{CIN}3 \), still a borderline significance was reached (\( P < 0.1 \)). The increase in sensitivity by combined testing compared to cytology alone may be misleading because improvements in sensitivity would be expected by adding a second test, even if the second test performed randomly with respect to disease identification. For this reason, expected performance characteristics for cytology combined with a random test were computed to determine if any gain in test performance from the addition of the second test (hrHPV testing) was greater than that expected if the second test offered no diagnostic information (Franco and Ferenczy, 1999). By comparing the sensitivity and NPV of cytology and hrHPV combined screening to cytology with a random test, no different significance levels were revealed as when compared to cytology alone.

Cost-effectiveness studies and modelling studies show that cervical screening may become much more efficient in terms of decreasing numbers of false-negative and false-positive smears, if a test is used with a substantial higher sensitivity and long-term NPV than conventional cytology (Canfell et al., 2004). Negative test results in combined screening predicted that the future risk for lesions \( \geq \text{CIN}3 \) was very low: The higher sensitivity for lesions \( \geq \text{CIN}3 \) and the long-term NPV for lesions \( \geq \text{CIN}3 \) of hrHPV testing in combination with classical cytology and of sole hrHPV testing show that these could be such a test.

The use of hrHPV testing in cervical screening could lead to several different screening strategies, including combined cytology and hrHPV testing, or primary screening by hrHPV with cytology reading only of women tested hrHPV positive. In addition, the high sensitivity and NPV of hrHPV testing opens possibilities for longer screening intervals with still acceptable rates of incipient lesions. Modelling studies and confirmation of our results in larger studies will help to clarify this discussion and to devise more efficient cervical screening strategies.

**ACKNOWLEDGEMENTS**

We thank NF Fransen-Daalmeijer, R Pol and M Verkuyten for the molecular technical work. We thank J Berkhof and S Bulk for their helpful comments on the manuscript, and AJC van den Brule for his support in the initial phase of this study. We gratefully acknowledge the assistance of the general practitioners in Amstelveen for enrolling the women in the study.

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**Human papillomavirus testing in cervical screening**

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Chapter 3

POBASCAM, a population-based randomised controlled trial for implementation of high-risk HPV testing in cervical screening;

Design, methods and baseline data of 44,102 women


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

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Cytological cervical screening is rather inefficient because of relatively high proportions of false negative and false positive smears. To evaluate the efficiency of high-risk human papillomavirus (hrHPV) testing, by GPS+/i6+ PCR-enzyme immunoassay (EIA), in conjunction with cytology (Intervention Group) to that of the classical cytology (Control Group), we initiated the Population Based Screening Study Amsterdam (POBASCAM). POBASCAM is a population-based randomized controlled trial for implementation of hrHPV testing in cervical screening. The outcome measure is the proportion of histologically confirmed ≥CIN3 lesions in each study arm up to and including the next screening round after 5 years. We present the design, methods and baseline data of POBASCAM. When, in the next 5 years, the follow-up will be completed, the data obtained will be used in model studies, including a cost-effectiveness study, to advise the Dutch Ministry of Public Health in deciding whether cervical screening should be based on combined hrHPV and cytology testing instead of cytology alone. Between January 1999 and September 2002, 44,102 women (mean age = 42.8 years; range = 29–61) that participated in the regular Dutch screening program were included in our study. In the Intervention Group the distribution of cytology and hrHPV by cytology class was as follows: normal cytology 96.6% (3.6% hrHPV positive); borderline and mild dyskaryosis (BMD) 2.5% (34.6% hrHPV positive); and moderate dyskaryosis or worse (>BMD) 0.8% (88.3% hrHPV positive), i.e., 0.4% moderate dyskaryosis (82.9% hrHPV positive), 0.3% severe dyskaryosis (92.5% hrHPV positive), 0.1% carcinoma in situ (95.2% hrHPV positive), <0.1% suspected for invasive cancer. The presence of hrHPV was age-dependent, decreasing from 12.0% at 29–33 years to 2.4% at 59–61 years. Among women with a positive hrHPV test, the prevalence of BMD was age-dependent ranging from 20.2% at 29–33 years to 7.8% at 54–58 years. In contrast, the risk of >BMD of 13.7% among women with a positive hrHPV test was not age-dependent. Our study indicates that large-scale hrHPV testing by GPS+/i6+ PCR-EIA in the setting of population-based cervical screening is practically feasible, is accepted by both participating women and general practitioners and yields highly reproducible results.

Key words: human papillomavirus; cytology; screening; cervical intraepithelial neoplasia; randomized controlled trial; age

Cervical screening by cytology in population-based screening programs has contributed substantially to the decrease in the incidence and mortality of cervical cancer.1–4 Even in countries with well-organized, quality controlled population-based screening programs, however, the proportion of false positive and false negative smears is at least 10%.5,6 In most screening programs a 3-way triage is used on the basis of the cytological classification. The large majority of women with a normal smear are advised to have the smear repeated at the next screening round. The interval between screening rounds varies from 1 year in Germany and the United States, 3 years in the United Kingdom and Sweden or 5 years in Finland and The Netherlands.7 The small group of women with serious cytomorphological abnormalities, i.e., moderate dyskaryosis or worse (abbreviated as >BMD, meaning “worse than borderline or mild dyskaryosis”; comparable to HSIL; Fig. 1), are referred for colposcopically directed biopsies (ColpoBx). A relatively large group of women, however, have minor abnormalities, i.e., borderline and mild dyskaryosis (BMD, comparable to ASCUS/LSIL; Fig. 1). The policy to repeat the smear for women with minor abnormalities is quite a burden because only 10% of them have or will develop clinically relevant lesions, i.e., cervical intraepithelial neoplasia Grade 3 (CIN 3), the most advanced precursor lesion for cervical carcinoma or worse.8,9 In the screening programs ongoing currently in Europe, women with BMD are only referred for ColpoBx when a repeated smear after 6 or 18 months is read as BMD or worse. If women with BMD who are at risk for CIN 3 or worse (>CIN 3) could be identified with a new test, a more efficient referral strategy could be devised. Such a test could involve high-risk human papillomavirus (hrHPV) detection because the presence of hrHPV is necessary for the development and maintenance of cervical intraepithelial neo-
In addition, women with hrHPV positive, cytology alone (Control Group). According to the guidelines, 5 women after enrollment in our study. In this way, in each study arm all historically confirmed lesions ≥CIN 3 up to and including those detected at the next screening round will be included in the final analyses.

The objective of this trial is to evaluate whether the efficiency of screening is improved by the addition of hrHPV testing to cytology by testing 2 major hypotheses. The first hypothesis concerns the possibility of a prolonged screening interval for women with normal cytology and a negative hrHPV test. A risk of lesions ≥CIN3 of 0.5–0.8% in the time period of 5 years (until the next screening round in the Dutch cervical screening program) for women with normal cytology is accepted currently by health authorities and professionals. We hypothesize that for women with normal cytology and a negative hrHPV test at baseline, this risk is much lower than the accepted risk. The screening interval for them, therefore, might be prolonged. This first hypothesis will be evaluated by testing whether the proportion of histologically confirmed ≥CIN 3 lesions among women with normal cytology and a negative hrHPV test at baseline (Group A.1 in Fig. 2), is less than that among women with normal cytology not hrHPV tested (Group D in Fig. 2).

The second hypothesis concerns the possibility of a reduced number of referrals for colposcopic examination for women with BMD without missing histologically confirmed ≥CIN 3 lesions. This will be evaluated by testing whether, during follow-up of up to 5 years, the proportion of lesions ≥CIN 3 is less in women with BMD of group B.1 B.2.1 compared to those of group E.1 (Fig. 2). The proportion of ≥CIN3 in women with BMD and a negative hrHPV test at baseline (group B.1) and women with BMD at baseline and hrHPV clearance at 6 months (group B.2.1), should be less than that among women with BMD at baseline and normal cytology at 6 months as screened by classical cytology (group E.1).

**Triage of women with normal cytology**

Women assigned to the Control Group were advised according to the current guidelines for cervical screening in The Netherlands (Fig. 2). The advice was based on cytology results alone (hrHPV test result blinded). According to the guidelines, 5 women after enrollment in our study. In this way, in each study arm all historically confirmed lesions ≥CIN 3 up to and including those detected at the next screening round will be included in the final analyses.

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for ColpoBx. In case of BMD/hrHPV− at 18 months, or normal cytology/hrHPV− at 18 months, women were not recalled until the next screening round.

Triage of women with BMD

Women with BMD were advised to repeat the tests after 6 and 18 months. In the Control Group, those who still had BMD or worse after either 6 or 18 months were referred to a gynecologist for ColpoBx. Women with regression to normal cytology after 18 months will not be recalled until the next screening round. In the Intervention Group, women who had BMD/hrHPV− at 6 months, or BMD/hrHPV− or normal cytology/hrHPV− at 18 months, were referred for ColpoBx. In case of BMD/hrHPV− or normal cytology/hrHPV− at 18 months, women will not be recalled until the next screening round.

Triage of women with >BMD

In the Intervention and Control Groups, women with >BMD detected at any time (i.e., at baseline or after retesting because of abnormal cytology in both groups or retesting because of a positive hrHPV test in the Intervention Group) were referred directly for ColpoBx. Referrals because of >BMD were always independent of the hrHPV test result.

Randomization, referral and follow-up

Women were randomly 1:1 assigned using the computer’s random number generator to the Intervention or Control Group at the time of receiving their administrative data in the central study database. Randomization was independent of cytology or hrHPV test result. Women could be referred for ColpoBx to one of about 40 gynecologists in 4 regional and 1 academic hospital. The gynecologists, who had been informed about POBASCAM, carried out colposcopy and colposcopically-directed biopsy for histological examination according to standardized guidelines of the Dutch Society of Obstetrics and Gynaecology. Histological biopsies were only taken when cervical abnormalities were seen, regardless of the hrHPV status. Eventually, women were treated according to standard protocols. Follow-up data are supplied by the 4 pathology laboratories participating in this trial via the Dutch Pathologisch Anatomisch Landelijk Gedecentraliseerd Archief (PALGA, the Dutch nationwide network and registry of histological and cytopathology).

Outcome measures

The primary outcome measure of POBASCAM is the proportion of histologically confirmed ≥CIN 3 lesions found during the time span from intake up to and including the next screening round (i.e., in 5 years). Because women with normal cytology at the next screening round will not be referred for ColpoBx and therefore will not have a histological endpoint, it will be assumed that no CIN lesions are present. This policy complies with standard cervical screening. Parameters obtained will include progression and regression of cytology diagnosis, clearance and acquisition of hrHPV and the number of referrals for ColpoBx to a gynecologist.

Recruitment and enrollment procedures

According to the Dutch cervical cancer-screening program, women between 30 – 60 years of age are invited every 5 years to have a Pap smear. The invitations for the regular screening program are coordinated by the District Health Authority (DHA). Together with the invitation for the regular screening program, women were informed about POBASCAM. Principally, women were invited by their general practitioner. Women not registered at any general practitioner were invited directly by the DHA. Women were eligible when they lived in a defined semi-urbanized region demarcated according to the DHA Southwest of Amsterdam, were between 30 – 60 years of age, were invited for the regular population-based screening program having a uterus in situ and were able to...
and willing to give written informed consent for our study. Women were excluded from analysis if they had abnormal cytology or a CIN lesion within a period of 2 years preceding enrollment. The 2-year period was chosen because according to Dutch guidelines during that time, smears could be repeat smears of an indicative smear in the previous 2 years. Women were also excluded for analysis when the sample taken for hrHPV testing at baseline was lost. When inadequate smears were repeated (from 6 weeks–6 months) the adequate smear was chosen as intake smear. The study was approved by the Medical Ethics Committee of the VU University Medical Center (nr 96/103A), and the Ministry of Public Health (VWS nr 326 650).

Education of general practitioners
A total of 242 general practitioners participated in our study. They were invited to attend postgraduate medical education courses and received written information (including a 22-page booklet) pertaining to the issue of answering frequently asked questions of participating women. An information help desk was also available for general practitioners and participating women.

Cytology
Cervical smears were taken by the general practitioner or their assistant using a Cervex-Brush® or a cytobrush. After making a conventional smear for cytological examination, the brush was placed in a vial containing collection medium (i.e., 5 ml PRS and 0.5% thiomersal) for hrHPV testing. Cervical smears were classified according to the CISOE-A classification by cytotecnologists and abnormal smears were reviewed by experienced cytopathologists (standard way of cervical screening in the nationwide screening program). For cervical screening, a detailed quality control program has been defined by the Netherlands Society for Pathology and implemented at the national level. The CISOE-A classification (in Dutch KOPAC-B) is the standard classification used in the Netherlands (Fig. 1). The comparison of this classification with the Bethesda 2001 classification is given in Figure 1. For our study, the smears were classified in either 1 of 4 participating regional pathology laboratories (Department of Pathology, Spaarne Ziekenhuis, Heemstede; Leiden Cytology and Pathology Laboratory, Leiden; Stichting PA Laboratorium Kenmerland, Haarlem; Department of Pathology, VU University Medical Center, Amsterdam).

Human papillomavirus testing
All women, including the women in the Control Group, have been hrHPV tested at the time of receipt of the HPV sample and all hrHPV testing has been carried out on a daily basis on freshly received material. All hrHPV tests were carried out independent of Pap smear reading in the Department of Pathology (Unit Molecular Pathology, VU University Medical Center). The vials containing the brush in collection medium were sent daily to the molecular pathology laboratory by courier or by mail. A duplicate GP5+/6+ PCR-EIA test was done as described previously. A mixture of probes for the detection of 14 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) was used. When the duplicates gave inconsistent results (1%), a repeat duplicate test was carried out and only samples with at least 2 positive tests were ultimately scored hrHPV positive. All the cases with these inconsistent duplicates involved smears with optical density values around the cut-off point, which is routinely used for this test (3 times the optical density of a series of blanks), indicating a relatively low viral load. The final test result was hrHPV positive or hrHPV negative.

Data processing
Data processing involved a central study database, 4 administrative databases (one in each participating pathology laboratory), and a database holding the hrHPV PCR-EIA test results. Between the central study database and the administrative databases, data were exchanged every month automatically by E-mail in a closed and secure computer network. Authorized hrHPV test results, as registered in the database holding the hrHPV PCR-EIA test results, were automatically copied to the central study database. Patient data and hrHPV test results were matched in the central study database. Patient data, cytology test results, as well as final hrHPV test results (either positive or negative for the Intervention Group or blinded for the Control Group) were manually entered in the administrative databases. Each laboratory had 2 coordinating cytotechnologists who generated a final trial advice according to the study protocol.

Statistical methods
A total sample size of 44,000 women was chosen to detect significant differences at a level of p = 0.05, 2-sided, with a power of 0.80, according to assumptions at the time the trial was designed (1996) of a prevalence for BMD of 14% and a prevalence for normal cytology of 84.5%. Women are invited for the regular screening program according to years of birth, every 5 years in the year they become 30, 35, up to 60 years of age. The first screening could be at 29 or 30 years of age. Therefore, the chosen age categories were 29–33, 34–38, etc. To assess the relation between hrHPV status and age categories and between cytology subclasses and age categories, logistic regression and multinominal regression methods were used. Odds ratios (OR) and predictive values were estimated from two-by-two tables. All analyses were carried out using SPSS software version 9.

RESULTS
Enrollment and age
Between January 1999 and September 2002, 49,220 women participated in cervical screening and were eligible for the POBASCAM study. A total of 44,938 women (91.3%) were enrolled. The major reason for women not to be enrolled in our study was largely of logistic origin at the general practitioner’s. The proportion of women who refused to participate in POBASCAM was very low (0.3%). After randomization, 22,420 women were allocated to the Intervention Group and 22,518 to the Control Group. In the Intervention Group, 216 women were excluded from analysis because of abnormal cytology or a CIN lesion within 2 years preceding enrollment. Another 7 women were excluded because of a previous hysterectomy and 201 women because of a missing hrHPV test result at baseline. For the same reasons, 217, 9 and 186 women, respectively, from the Control Group were excluded from analysis. Ultimately, the Intervention and Control Groups consisted of 21,996 and 22,106 women, respectively (Fig. 2). The Intervention and Control Groups were comparable for absolute numbers at enrollment, baseline cytology results, age and age distribution. The mean age in both groups was 42.8 years (range = 29–61). Table 1 shows the baseline cytology and baseline hrHPV test results for the Intervention and Control Group.

Cytology
Cytology in both arms showed comparable results (Table 1). The Intervention Group showed 0.2% inadequate smears, 96.6% normal, 2.0% borderline dyskaryotic, 0.5% mildly dyskaryotic, 0.4% moderately dyskaryotic, 0.3% severely dyskaryotic, 0.1% carcinoma in situ and <0.1% invasive carcinoma smears. The Control Group showed 0.2% inadequate smears, 96.5% normal, 2.0% borderline dyskaryotic, 0.4% mildly dyskaryotic, 0.4% moderately dyskaryotic, 0.3% severely dyskaryotic, 0.1% carcinoma in situ and <0.1% invasive carcinoma smears.

hrHPV
hrHPV testing by GP5+/6+ PCR-EIA in this large-scale screening setting turned out to be practically feasible, and with hrHPV testing being carried out in duplicate, we observed good reproducibility. As analyzed in the Intervention Group, a more
severe cytology diagnosis was associated with an increased rate of hrHPV positivity ($p$-trend < 0.01) (Table I). Among the women with normal cytology 3.6% had a positive hrHPV test. This percentage was 27.4% for women with borderline dyskaryosis and 64.4% for women with mild dyskaryosis. Combining these women into one group of women with BMD showed a positive hrHPV test of 34.6%. In the combined group of women with BMD, 88.3% had a positive hrHPV test, i.e., an hrHPV positivity rate in moderate dyskaryosis of 82.9%, in severe dyskaryosis of 92.5%, in suspected for carcinoma in situ of 95.2% and in suspected for invasive cancer of 100%.

**Histological diagnoses at baseline for women with >BMD**

Because women with >BMD at baseline are directly referred for ColpoBx irrespective of the hrHPV test result, cytology and hrHPV results could be related to histologically confirmed diagnoses (Fig. 3). The yield of histologically confirmed lesions $\geq$CIN 3 was comparable for the intervention and the Control Group, 92/171 and 92/179 respectively. Among the 171 women with >BMD in the Intervention Group, 151 women had a positive hrHPV test and for 23 women histologically follow-up was not yet available, leaving 128 analyzable women in the Intervention Group with >BMD, a positive hrHPV test and histology. Eighty-nine had a histologically confirmed lesion $\geq$CIN 3 including 5 squamous cell carcinomas (SCC). Likewise, for 5 of 20 women in the Intervention Group with >BMD and a negative hrHPV test, histological follow-up was not yet available, leaving 15 women in the Intervention Group with >BMD, a negative hrHPV test and histology. Three of the 15 had a histologically confirmed CIN 3 lesion (no SCCs). Thus, among women with >BMD, the proportion of histologically confirmed lesions $\geq$CIN 3 was associated with an increased rate of hrHPV positivity ($p$-trend < 0.01) (Table I). Among the women with normal cytology 3.6% had a positive hrHPV test. This percentage was 27.4% for women with borderline dyskaryosis and 64.4% for women with mild dyskaryosis. Combining these women into one group of women with BMD showed a positive hrHPV test of 34.6%. In the combined group of women with >BMD, 88.3% had a positive hrHPV test, i.e., an hrHPV positivity rate in moderate dyskaryosis of 82.9%, in severe dyskaryosis of 92.5%, in suspected for carcinoma in situ of 95.2% and in suspected for invasive cancer of 100%.

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with a positive hrHPV test (OR = 9.1, 95% CI = 2.4–34.2; Fig. 3). The sensitivity and negative predictive value for histologically confirmed lesions ≥CIN 3 of hrHPV testing under the condition of having >BMD were 96.7% and 80.0%, respectively.

**hrHPV prevalence in relation to age and cytology class**

For all women in the Intervention Group taken together, the prevalence of hrHPV decreased significantly with increasing age from 12.0% in the age category of 29–33 years, 6.8% at 34–38 years, 4.4% at 39–43 years, 3.1% at 44–48 years, 2.5% at 49–53 years, 2.6% at 54–58 years, to 2.4% at 59–61 years (p-trend < 0.01). In women with normal cytology, the hrHPV prevalence decreased from 8.3% in younger women (29–33 years of age) to 2.0% in older women (59–61 years of age; p-trend < 0.01). In Table II, classification of cytology is stratified according to age and hrHPV test result. Among women with a negative hrHPV test, small but age-independent proportions of about 0.1% had >BMD and about 1.7% had BMD. For women with a positive hrHPV test, the risk of >BMD was 13.7% (151/1102), which was independent of age (p-trend = 0.26, range = 13.7% in women 29–33 years, 11.6% in women 59–61 years). Among hrHPV positive women, however, the risk of BMD of 16.8% decreased with increasing age, (p-trend < 0.01, range = 20.2% in women 29–33 years, 7.8% in women 54–58 years).

**DISCUSSION**

The POBASCAM trial (n = 44,102) shows that hrHPV testing by GPS+/+ PCR EIA is practical in a large-scale, population-based routine primary screening setting. With hrHPV testing being carried out in duplicate we observed good reproducibility. The participation rate among women attending the nationwide cervical screening at one of the general practitioners participating in the study was very high. Of the eligible women, 91.3% was enrolled in the study. In most cases, women were not enrolled in our study due to logistic reasons at the general practitioner’s. The proportion of women not consenting to participate in POBASCAM was low (0.3%). This participation rate is in accordance with other studies.19,20 Because the general practitioners had to answer questions of women about hrHPV testing and the POBASCAM study, it was of essential value to provide them with expert advice. This was done by concise written information (a 22-page booklet), by postgraduate medical education courses including the issue of answering frequently asked questions of participating women and by providing the possibility of asking immediately additional information at a help-desk.

The POBASCAM study will yield parameters to be used in modelling studies, including a cost-effectiveness analysis. These studies will be done to estimate the most efficient algorithm for the detection of ≥CIN 3 lesions in well-organized population-based cervical screening with less inconvenience for the women concerned. Parameters to be estimated include the age-dependent prevalence of hrHPV, hrHPV clearance and acquisition, the risk of having or developing lesions ≥CIN 3 stratified to cytology class among hrHPV positive and hrHPV negative women, the number of regressive CIN lesions among women more closely followed than the standard 5-years interval of regular screening and the sensitivity and specificity of hrHPV testing. The sensitivity and specificity of cytology can be estimated closely using the number of lesions ≥CIN 3 detected among women in the Intervention Group with normal cytology and a (persistently) positive hrHPV test because the vast majority (about 90%) of women with lesions ≥CIN 3 and a false-negative Pap-smear will have persistently positive hrHPV tests. Hence, in the Intervention Group it can be expected that about 90% of the women with false negative cytology will be detected (Groups A.2.3, A.2.2.2 and A.2.2.3 in Fig. 1). In the POBASCAM study women with normal cytology and a positive hrHPV test are retested after 6 and 18 months. These intervals were chosen in relation to the mean clearance time of hrHPV of about 1 year.16

The sample size of 44,000 women was chosen according to assumptions at the time when the trial was designed. In 1996, however, the guidelines of the Dutch regular screening program have changed, by introducing the CISOE-A classification. As a consequence, the proportion of women categorized as BMD decreased from 11.4–2.6%, whereas the subcategory of women with normal cytology increased from 84–96%.31,32 In the new CISOE-A classification the diagnosis of "borderline changes" is more strictly defined. Similar changes were made in the new guidelines of Bethesda 2001 Classification.32 Before the introduction of the new CISOE-A Classification, a total number of about 5,000 women with BMD would have been diagnosed among 44,000 women screened. To obtain a similar number of women with BMD after the introduction of CISOE-A, the study should have been based on a total of about 190,000 women screened. Although this could mean that testing of the hypothesis concerning referral of women with BMD smears might not reach statistical significance, the parameters obtained with the POBASCAM study will have good accuracy for modelling studies.

From the outset of the POBASCAM study some unavoidable detection bias can be expected when comparing the number of ≥CIN 3 lesions in Group A.1 vs. Group D (Fig. 2). In the current Dutch primary cervical screening program (women age 30–60 years, screening interval = 5 years), a very low risk for interval cervical carcinoma is accepted by health authorities and professionals, which is related to an incidence of lesions ≥CIN 3 of 0.5–0.8% per screening interval of 5 years. The Medical Ethics Committee and the Ministry of Public Health did not give permission for additional cytology and hrHPV testing or ColpoBx for women with normal cytology with that accepted low risk (Groups A.1 and D in Fig. 2). They further reasoned that the number of

---

**TABLE II** — AGE-RELATED PREVALENCE OF CYTOLOGY CLASSIFICATION ACCORDING TO hrHPV STATUS OF 21,950 WOMEN IN THE INTERVENTION GROUP

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Normal</th>
<th>BMD</th>
<th>hrHPV+</th>
<th>&gt;BMD</th>
<th>Normal</th>
<th>hrHPV-</th>
<th>&gt;BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>29–33</td>
<td>246 (66.1)</td>
<td>75 (20.2)</td>
<td>51 (13.7)</td>
<td>2.713 (98.2)</td>
<td>48 (1.7)</td>
<td>2 (0.1)</td>
<td></td>
</tr>
<tr>
<td>34–38</td>
<td>208 (66.9)</td>
<td>61 (19.6)</td>
<td>42 (13.5)</td>
<td>4.180 (98.3)</td>
<td>67 (1.6)</td>
<td>4 (0.1)</td>
<td></td>
</tr>
<tr>
<td>39–43</td>
<td>95 (69.9)</td>
<td>18 (13.2)</td>
<td>23 (16.8)</td>
<td>3.307 (98.0)</td>
<td>64 (1.9)</td>
<td>4 (0.1)</td>
<td></td>
</tr>
<tr>
<td>44–48</td>
<td>67 (69.8)</td>
<td>15 (15.6)</td>
<td>14 (14.6)</td>
<td>3.324 (98.2)</td>
<td>58 (1.7)</td>
<td>2 (0.1)</td>
<td></td>
</tr>
<tr>
<td>49–53</td>
<td>57 (74.0)</td>
<td>11 (14.3)</td>
<td>9 (11.7)</td>
<td>2.947 (97.8)</td>
<td>64 (2.1)</td>
<td>3 (0.1)</td>
<td></td>
</tr>
<tr>
<td>54–58</td>
<td>52 (81.3)</td>
<td>5 (7.8)</td>
<td>7 (10.9)</td>
<td>2.136 (98.4)</td>
<td>31 (1.4)</td>
<td>4 (0.2)</td>
<td></td>
</tr>
<tr>
<td>59–61</td>
<td>38 (88.4)</td>
<td>0 (0.0)</td>
<td>5 (11.6)</td>
<td>1.875 (99.0)</td>
<td>17 (0.9)</td>
<td>1 (0.1)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>763 (69.4)</td>
<td>185 (16.8)</td>
<td>151 (13.7)</td>
<td>20.482 (98.2)</td>
<td>349 (1.7)</td>
<td>20 (0.1)</td>
<td></td>
</tr>
</tbody>
</table>

*p-value*  
Reference p < 0.01 p = 0.26

*p* for trend in age category in multinominal regression analyses.

---

1Values are n (%). BMD, borderline and mild dyskaryosis; >BMD, cytology worser than mild dyskaryosis; hrHPV+, hrHPV positive; hrHPV−, hrHPV negative. 46 women with inadequate cytology are excluded from this table. Mean age 42.8 years (range 29–61).2Percentages of women with normal cytology, BMD or >BMD in hrHPV positive group for each age category.3Percentages of women with normal cytology, BMD or >BMD in hrHPV negative group for each age category.
ColpoBx and the results to be expected do not warrant the strain for the women involved. A similar type of bias can be expected when comparing the number of ≥CIN 3 lesions in Group B.1 + B.2.1 vs. Group E.1 (Fig. 2). According to our study design, these women were retested at 6 and 18 months of follow-up. They were referred for ColpoBx if retesting showed either >BMD or a positive hrHPV test. However, women with BMD and a negative hrHPV test were not referred to wait to the next screening round, which is after 3.5 years. This follows from studies from Manos et al. and Clavel et al. and from our own experience (follow-up up to 4.3 years). From these studies it can be calculated that the risk of ≥CIN 3 of women with a negative hrHPV test, either in conjunction with normal cytology or BMD is very low when tested additionally after 6 and 18 months (Group B.1 + B.2.1 in Figure 2). This risk is expected to be at least as low as that for women with normal cytology in the standard Dutch nationwide screening program with an interval of 5 years. The magnitude of these biases in detecting histologically confirmed lesions ≥CIN 3 up to and including those found at the next screening round can still be estimated when the results of hrHPV testing in the Control Group can be taken into account and data of the Control Group can be compared to those of the Intervention Group.

The cytology baseline results of our study are comparable with the cytology results of the Dutch nationwide screening program in the time period of recruitment and with the results reported in one region of the Dutch nationwide screening program. Moreover, our baseline hrHPV prevalence of 5.0% is in accordance with international literature. Two studies in the setting of population-based screening among women with a comparable mean age showed similar hrHPV prevalences. In other studies, higher prevalences of hrHPV were found, which is probably related to the study population, age or the hrHPV test used. Using HClI in a hospital-based population, Clavel et al. found an hrHPV prevalence of 15%. Among younger women (mean age = 25 years) Kulasingam et al. found an hrHPV of 18%, and among women with a mean age of 30 years, Ratnam et al. found a prevalence of 10%. Because hrHPV is considered to be the predominant agent for the development of cervical carcinoma and its precursor lesions, we analyzed the prevalence of BMD and >BMD among women screened according to hrHPV status.

In women with a negative hrHPV test the prevalences of BMD or >BMD were low and independent of age. In women with a positive hrHPV test, the prevalence of >BMD of 13.7% (or about 1 in 7) was also independent of age, i.e., women with an hrHPV infection have a flat risk of having severe cervical lesions. In contrast, for women with a positive hrHPV test the prevalence of BMD was age-dependent, which suggests that most BMD lesions reflect a cytopathological effect of primary hrHPV infection. Naturally, with increasing age, the chance of contracting a primary hrHPV infection decreases that could explain the relative decrease in BMD prevalence.

Of special interest are the 3 cases of histologically confirmed ≥CIN 3 lesions among women with >BMD and a negative hrHPV test. After additional analyses, one sample shown to be β-globin negative and after DNA-isolation this sample turned out to contain viral integration with disruption of the L1 region that is recognized by the E7 specific PCR, another sample shown to be positive for type 66, indicating viral integration with disruption of the L1 region that is recognized by the E7 specific PCRs. The third sample remained hrHPV negative after additional analyses with E7 type specific PCRs. Because women with >BMD are indirectly referred for ColpoBx, irrespective of hrHPV status, hrHPV prevalence among these women does not have clinical consequences.

Our large-scale study indicates that hrHPV testing by GSP/6+/PCR EIA in the setting of population-based cervical screening is practically feasible, is accepted by both participating women and general practitioners and yields highly reproducible results.

ACKNOWLEDGEMENTS

We gratefully acknowledge the work of the 242 general practitioners and their assistants, the cytotechnologists and administrators from the 4 regional laboratories, the cytotechnologists and the molecular technicians of the pathology department of VU University Medical Center, Medial Haarlem, the District Health Authority Amstelveen, DIH Kennemerland-Haarlemmermeer e.o., and PALGA, for their work and support in our study. We especially thank, in alphabetical order, R. van Andel, B. Bakker, I. Barendse, R. van Beek, J. Belien, A. Bizee, J. ter Borg, S. Bulk, M. van Casteren, P. van Dijken, N. Fransen-Daarstmeijer, A. van der Geest, A. Groothuismink, F. Hendrikse, H. van Keep, M. van der Laan, M. Lettink, M. Meurs, J. Pleisters, R. Pol, S. van Schaick, D. Schumacher, F. Stuart-Vogelsang and M. Verkuyten, for their technical assistance. We thank H. Berkhof for his statistical advice. The study is funded by ZON, Zorg Onderzoek Nederland (Netherlands Organisation for Health Research and Development: grant 30-0522/20).

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IMPLEMENTATION OF hrHPV TEST IN CERVICAL CANCER


Chapter 4

Implementation of Human Papillomavirus testing in cervical screening without a concomitant decrease in participation rate

Nicole W. J. Bulkmans, Saskia Bulk, M. Sigrid Ottevanger, Lawrence Rozendaal, Simone M. Hellenberg, Folkert J. van Kemenade, Peter J. F. Snijders, A. Joan P. Boeke, and Chris J. L. M. Meijer

*Journal of Clinical Pathology* 2006; 59(11): 1218-1220
SHORT REPORT

Implementation of human papillomavirus testing in cervical screening without a concomitant decrease in participation rate


Adding high-risk human papillomavirus (hrHPV) testing to screening increases the efficacy of cervical screening programmes. However, hrHPV testing may result in a lower participation rate because of the perceived association with sexually transmitted infections. We describe how testing for hrHPV was added to cervical screening in the POBASCAM trial.

In The Netherlands, the POBASCAM trial evaluates the effectiveness of adding hrHPV testing to cervical screening by cytology within the confines of the regular population-based screening programme. Here, we describe which measures were taken during implementation of the hrHPV test to prevent lower participation in cervical screening. We evaluated the effectiveness of these measures by comparing participation rates before and after the introduction of hrHPV testing, and tabulated frequently asked questions during the trial.

METHODS

The cervical screening programme in The Netherlands is a population registry-based programme, inviting women aged 30–60 years seven times at 5-year intervals. The POBASCAM trial is a population-based randomised, controlled trial to evaluate the efficacy of screening using hrHPV testing. Participants were randomised either to a control group receiving repeat and referral recommendations based on cytology diagnosis only (ie, without receiving hrHPV test results), or to an intervention group receiving both cytology diagnosis and hrHPV test results. Baseline results have been described previously.

Between 1999 and 2002, we included a total of 44 102 women invited for population-based cervical screening in the trial area. All general practitioners in the trial area were invited to contribute participants to the POBASCAM trial. Women received information on the trial and the nature of hrHPV infections, highlighting the lifetime prevalence and clearance rate of infections. Contributing general practitioners sampled cervical material for both a smear and hrHPV testing in screened women, and informed participants about the results of their test. Before and during the trial, contributing general practitioners were offered postgraduate courses on hrHPV and its relationship with cervical cancer. The information was aimed to be sufficient to answer any question of a participant in one consultation. If necessary, study coordinators (NWJB and SB) could be contacted by telephone by general practitioners and participants for further explanation. All questions were registered.

The Registry of the District Health Authority on participation rates was complete from 1997 onwards for individual rates per general practitioner from 2000 onwards. Participation rates were defined as the ratio of the number of screening smears to the number of invitations. The participation rates in the periods before (1997–8, cytology only) and during the enrolment phase of POBASCAM (1999–2002, hrHPV and cytology combined) were compared for general practitioners in the study area who contributed to the POBASCAM trial and for non-contributing general practitioners. Ratios were compared using the χ² analysis and test for trend. p Values of ≤0.05 were considered significant.

RESULTS

The participation rate of the cervical screening programme did not decrease after implementation of an hrHPV test in 1999 (table 1A).

The participation rate in the cervical screening programme was 58.7% (range 51.6–63.2%) in 1997–8 in the trial area and did not decrease after implementation of the hrHPV test in 1999–2002 to 61.4% (range 60.7–62.3%; p for trend = 0.267). Moreover, participation rate was higher for contributing GPs than for non-contributing general practitioners (66.8% v 52.7% respectively; p < 0.001; table 1B).

We registered telephone consultations received throughout the intake phase of the trial; there were 51 calls of participants and 92 calls of contributing general practitioners on behalf of participants. Table 2 lists the most frequently asked questions and respective answers provided by the study coordinators. Mostly, questions were related to the viral nature and sexual transmission of hrHPV and seemed elicited on receiving a test result in the non-blinded arm of the trial with an advice requesting earlier repeat tests or more urgent referral advice for colposcopically directed biopsies.

Abbreviations: hrHPV, high-risk human papillomavirus; POBASCAM, POpulation-based SCreening study AMsterdam

Implementation of HPV testing

Table 1  Participation rates in cervical screening

<table>
<thead>
<tr>
<th>Year</th>
<th>Overall</th>
<th>Year</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Invitations</td>
<td>Smears</td>
<td>Participation rate</td>
</tr>
<tr>
<td>1997</td>
<td>31 534</td>
<td>16 263</td>
<td>51.6</td>
</tr>
<tr>
<td>1999</td>
<td>33 489</td>
<td>20 334</td>
<td>60.7</td>
</tr>
<tr>
<td>2001</td>
<td>34 204</td>
<td>21 316</td>
<td>62.3</td>
</tr>
</tbody>
</table>

B. Non-contributing and contributing general practitioners in hrHPV testing

<table>
<thead>
<tr>
<th>Year</th>
<th>Invitations</th>
<th>Smears</th>
<th>Participation rate</th>
<th>Invitations</th>
<th>Smears</th>
<th>Participation rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>10 573</td>
<td>5422</td>
<td>53.2</td>
<td>21 725</td>
<td>14 489</td>
<td>66.7</td>
</tr>
<tr>
<td>2001</td>
<td>13 153</td>
<td>6971</td>
<td>53.0</td>
<td>21 051</td>
<td>14 345</td>
<td>68.1</td>
</tr>
<tr>
<td>2002</td>
<td>12 923</td>
<td>6733</td>
<td>52.1</td>
<td>20 063</td>
<td>13 146</td>
<td>65.5</td>
</tr>
</tbody>
</table>

hrHPV, high-risk human papillomavirus.

*Non-contributing general practitioners; Contributing general practitioners.

DISCUSSION

Before initiating the POBASCAM trial, a survey conducted among 1551 Dutch women indicated that hrHPV testing would not interfere with participating in cervical screening. Indeed, the overall participation did not decrease after starting the trial. More remarkably, the participation rate was increased in contributing general practitioners compared with non-contributing general practitioners. Several explanations can be offered for this increase in participation rates. Firstly, contributing general practitioners were more motivated to achieve good participation rates. Unfortunately, the District Health Authority did not register participation rates stratified per general practitioner before 2000. Secondly, women participating in the POBASCAM trial were more motivated because of the possibility of more extensive testing.

All invited women received information by the health authorities about the trial, together with the invitation. In our study, a few participants contacted the study coordinators to obtain more information than was supplied routinely. FAQs dealt mainly with the viral nature and sexual transmission of hrHPV. Most questions by participants concerned the viral nature and transmission of hrHPV.

Table 2  Frequently asked questions in the POBASCAM trial regarding infections with hrHPV

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>How can an infection with hrHPV be treated?</td>
<td>Currently, there is no treatment for an hrHPV infection. Most hrHPV infections (80%) are cleared by the immune system itself. Should you have the virus and eventually have developed cervical abnormalities, the latter can be treated by your gynaecologist. Usually, after treatment of cervical lesions hrHPV can also no longer be detected. At the moment, prophylactic vaccines are being developed.</td>
</tr>
<tr>
<td>How can I have an hrHPV infection after 25 years of age?</td>
<td>Usually, the virus will be undetectable in 80% of all women after 1 1/2 to 2 years.</td>
</tr>
<tr>
<td>Will an hrHPV infection be cleared at the next test?</td>
<td>Yes, hrHPV is a very common virus. Up to 85% of all women will at some point in their life have experienced an hrHPV infection. hrHPV is sexually transmitted. We cannot, however, totally exclude other ways of transmission.</td>
</tr>
<tr>
<td>Is hrHPV a very common virus and how do you become infected?</td>
<td>hrHPV may be present in very low quantities under the level of detection, and harmless for your body. Now, many years later, the virus may be activated, possibly due to a weakened immune system. The virus may replicate and increase in quantity, and subsequently cause cervical lesions. So, you cannot deduct from a positive hrHPV test that hrHPV is acquired from recent extramarital contact.</td>
</tr>
<tr>
<td>What are the consequences of having an hrHPV infection?</td>
<td>Currently, there is no treatment for an hrHPV infection. Most hrHPV infections (80%) are cleared by the immune system itself. Should you have the virus and eventually have developed cervical abnormalities, the latter can be treated by your gynaecologist. Usually, after treatment of cervical lesions hrHPV can also no longer be detected. At the moment, prophylactic vaccines are being developed.</td>
</tr>
</tbody>
</table>

Take-home messages

- Overall participation in the screening programme did not decrease after the introduction of hrHPV testing.
- With the introduction of hrHPV testing in a screening programme, most questions by participants concerned the viral nature and sexual transmission of hrHPV.
- Attention has to be paid to give clear and consistent information about hrHPV to screened women and to general practitioners.

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Patient consent has been obtained for publication of this study.

Funding: This work was supported by grant 30-522 from the Health Research & Development Council of The Netherlands (ZonMw, formerly the Praveentiefonds).

Competing interests: None.

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**Both authors contributed equally to this manuscript.**

Ethical approval: This study was approved by the medical ethics committee of the University Medical Center, Amsterdam, The Netherlands (number 96/103A), and by the Ministry of Public Health, The Hague, The Netherlands (VWS number 328 650).

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Accepted 25 August 2005
Chapter 5

Prevalence of types 16 and 33 is increased in high-risk human papillomavirus positive women with cervical intraepithelial neoplasia grade 2 or worse

Nicole W.J. Bulkmans, Maaike C.G. Bleeker, Johannes Berkhof, Feja J. Voorhorst, Peter J.F. Snijders, and Chris J.L.M. Meijer

High-risk human papillomavirus (hrHPV) types are causally related to cervical cancer and its high-grade precursor lesions. The risk posed by the different hrHPV types for the development of cervical intraepithelial neoplasia grade 2 or worse (≥CIN2) needs to be established. Here, we present the hrHPV type-distribution in relation to cytology and histology for women participating in a cervical screening program. From 44,102 women who participated in a population-based cervical screening program in the Netherlands, 2,154 hrHPV positive women were included to determine the distribution of 14 hrHPV types by reverse line blotting of GP5+/6+ PCR products. For each HPV type, associations with cytology and histologically confirmed ≥CIN2 were measured by odds ratios. HPV types 16 and 33 were more prevalent in women, amongst those containing a single hrHPV type, with moderate dyskaryosis or worse (>BMD) than in women with normal cytology, but only in case of underlying ≥CIN2 (OR 4.10, 95% CI 2.98–5.64 and OR 2.68, 95% CI 1.39–5.15, respectively). Similar results were obtained for women with double infections (OR 3.29, 95% CI 1.61–6.75 and OR 4.37, 95% CI 1.17–16.34). Coexisting types did not influence the prevalence of ≥CIN2 in HPV 16 or 33 positive women. The increased prevalence of type 16 and 33 in hrHPV positive women with ≥CIN2, compared to women with normal cytology, suggests that infection with these types confers an increased risk for development of ≥CIN2. Distinguishing these types may therefore have implications for future cervical screening strategies.

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Key words: human papillomavirus; HPV 16; HPV 33; cervical screening; cervical intraepithelial neoplasia

A subset of human papillomavirus (HPV) types are considered to be causally related to cervical cancer and its high-grade precursor lesions. Although geographical variations exist in high-risk HPV (hrHPV) type distribution, HPV type 16 is by far the most predominant HPV type in cervical cancer, being found in 54.4% of cervical squamous cervical carcinomas (SCCs) worldwide and in 69.7% of these cases in Europe/North America. A recent meta-analysis, comparing high-grade squamous intraepithelial lesions (HSILs) with SCC, revealed that HPV 16, 18 and 45 were each more prevalent in SCC, whereas the reverse was the case for other hrHPVs, including HPV 31, 33, 52 and 58. This suggests that HPV 16, 18 and 45 have an increased potential to induce invasive carcinoma.

Testing of cervical scrapings for hrHPV types as a pool in adjunct to cervical cytology is becoming increasingly attractive as data are accumulating that this combined test increases the efficacy of cervical screening programs and triage policies for women with equivocal cervical smears. However, data on the distribution of hrHPV types in sufficiently large numbers of women in a population-based setting are lacking. This has hampered a comparative analysis of hrHPV type-specific prevalence between positive women with and without histologically confirmed ≥CIN2. Such comparisons may provide insight into a possible differential potential of hrHPV types to induce progression to ≥CIN2.

In this cross-sectional study we determined the hrHPV type-distribution in relation to cytology and underlying histologically confirmed ≥CIN2 amongst a total of 2,154 hrHPV positive women derived from 44,102 women who participated in a population-based cervical screening program.

Material and methods

Study population and data collection

From January 1999 until September 2002, a total of 44,102 women participating in the Population Based Screening Amsterdam (POBASCAM) trial (the Netherlands) were recruited from 242 general practitioners. POBASCAM was initiated to evaluate the efficacy of hrHPV testing in conjunction with cytology compared to that of sole classical cytology. The 2-armed trial was carried out within the setting of the regular Dutch nationwide cervical screening program in which women between 30–60 years of age are invited with screening intervals of 5 years. Further details about POBASCAM have been described previously. Informed consent was obtained from all participating women.

Cervical smears were taken using a Cervex brush® or a cyto-brush. After making a conventional smear for cytological examination, the brush was placed in a vial containing collection medium (i.e., 5 ml PBS and 0.5% thiomersal) for hrHPV testing. For cytology, smears were classified according to the CISOE-A classification, which can be translated easily to the Bethesda 2001 classification. Women with normal cytology and borderline of mild dyskaryosis (BMD; ASCUS/ASC-H/LSIL according to the Bethesda classification), different follow-up scenarios were followed for the 2 study arms. Women with a cervical cytology worse than BMD (>BMD; HSIL according to the Bethesda classification) were immediately referred to the gynecologist for colposcopy. Colposcopically directed biopsies for histological examination were taken and histologically classified as no CIN, CIN 1, 2 or 3 according to international criteria.

Detection of hrHPV DNA in the baseline scrapings was performed by GP5+/6+ PCR enzyme immunosassay, using a hrHPV

Abbreviations: BMD, borderline or mild dyskaryosis; >BMD, moderate dyskaryosis or worse; CIN, cervical intraepithelial neoplasia; ≥CIN2, cervical intraepithelial neoplasia grade 2 or worse; ≥CIN3, cervical intraepithelial neoplasia grade 3 or worse; CxCa, cervical carcinoma; HPV, Human Papillomavirus; hrHPV, high-risk Human Papillomavirus; HSIL, high-grade squamous intraepithelial lesions; ORs, Odds Ratios; POBASCAM, Population Based Screening Amsterdam; SCC, squamous cervical carcinoma.

Grant sponsor: ZON, Zorg Onderzoek Nederland (Netherlands Organisation for Health Research and Development; Grant number: 30-05220.

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Received 22 February 2005; Accepted 8 April 2005
DOI 10.1002/cncr.21210
Published online 17 May 2005 in Wiley InterScience (www.interscience.wiley.com).
A cocktail of 14 hrHPV types, i.e., 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68, GP5+/6+ PCR hrHPV positive cases were subsequently typed by reverse line blotting. Detailed protocols for these assays have been described elsewhere. HR HPV testing was carried out independent of histology and/or cytology results at the Department of Pathology, VU University Medical Center, Amsterdam.

Statistical analysis

Of the 44,102 enrolled women, 42,583 (96.6%) women had normal cytology, 1080 (2.4%) BMD and 350 (0.8%) >BMD. Another 89 (0.2%) women had inadequate grade. As reported earlier, the prevalence of hrHPV as detected by GP5+/6+ PCR was 3.7% (n=1,576) in cytologically normal women, 35.3% (n=381) in women with BMD, 89.4% (n=313) in women with >BMD and 4.5% (n=4) in women with inadequate cytology. Women who were positive for hrHPV by GP5+/6+ PCR but negative by reverse line blot typing were excluded from the analysis (n=116), leaving a total of 2,154 hrHPV positive women with adequate cytology for the analyses. These comprised 1,467 women with normal cytology, 374 with BMD and 313 with >BMD. The women with normal cytology formed the reference category in all analyses. Results for hrHPV types other than 16 were calculated after excluding hrHPV 16 positive women.

We first selected all women with a single hrHPV infection. For each hrHPV type, we examined whether the prevalence was different for women with >BMD compared to those with normal cytology. The degree of association was measured by the Mantel-Haenszel common odds ratio (OR). Data were stratified in age categories corresponding to the 5 years rounds in nationwide screening (i.e., 29–33, 34–38, 39–43, 44–48, 49–53, 54–58 and 59–63). Statistical significance of the association between prevalence of hrHPV type and cytology was assessed by Cochran’s Mantel-Haenszel test. The presence of an association between OR and age was tested by Bre-slow-Day’s test of homogeneity. We subsequently examined whether the elevated prevalence of an hrHPV type in >BMD could be attributed to the presence of an underlying histological cofounders ≥CIN2 lesion. The >BMD group was split into two groups using ≥CIN2 as threshold. Separate ORs were calculated for ≥CIN3, being the immediate precursor lesion of cervical cancer.

We repeated the analyses for women with a double or ≥triple hrHPV infection. Because sample sizes per age stratum were small and age was not associated with cytological grade for women with multiple infections, strata were pooled. Statistical significance of the ORs was assessed by Fisher-exact tests. For each hrHPV type, we also assessed whether the presence of particular combinations was associated with ≥CIN2 (Fisher-exact test). Finally, we selected all women that were positive for a certain hrHPV type and assessed whether the prevalence of ≥CIN2 was associated with single or multiple infections by OR.

A limitation of any cross-sectional observational study is that ORs may be affected by bias. By using only data from 1 population-based cohort and by controlling for age, we ruled out overt bias. To strengthen the interpretation of the ORs as reflecting an immediate precursor lesion of cervical cancer.

Results

Study subjects

Of the 2,154 hrHPV positive women, 1,467 had normal cytology, 374 BMD and 313 >BMD. The mean age per cytological

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| Odds Ratios significantly different from 1, as assessed by Cochran’s Mantel-Haenszel test, are indicated in bold. Results presented for hrHPV types other than 16 were calculated after excluding hrHPV 16 positive women.

Table 1. Prevalence and odds ratios for hrHPV types among hrHPV positive women with single infections by cervical lesion grade

Statistical analysis

Of the 44,102 enrolled women, 42,583 (96.6%) women had normal cytology, 1080 (2.4%) BMD and 350 (0.8%) >BMD. Another 89 (0.2%) women had inadequate grade. As reported earlier, the prevalence of hrHPV as detected by GP5+/6+ PCR was 3.7% (n=1,576) in cytologically normal women, 35.3% (n=381) in women with BMD, 89.4% (n=313) in women with >BMD and 4.5% (n=4) in women with inadequate cytology. Women who were positive for hrHPV by GP5+/6+ PCR but negative by reverse line blot typing were excluded from the analysis (n=116), leaving a total of 2,154 hrHPV positive women with adequate cytology for the analyses. These comprised 1,467 women with normal cytology, 374 with BMD and 313 with >BMD. The women with normal cytology formed the reference category in all analyses. Results for hrHPV types other than 16 were calculated after excluding hrHPV 16 positive women.

We first selected all women with a single hrHPV infection. For each hrHPV type, we examined whether the prevalence was different for women with >BMD compared to those with normal cytology. The degree of association was measured by the Mantel-Haenszel common odds ratio (OR). Data were stratified in age categories corresponding to the 5 years rounds in nationwide screening (i.e., 29–33, 34–38, 39–43, 44–48, 49–53, 54–58 and 59–63). Statistical significance of the association between prevalence of hrHPV type and cytology was assessed by Cochran’s Mantel-Haenszel test. The presence of an association between OR and age was tested by Bre-slow-Day’s test of homogeneity. We subsequently examined whether the elevated prevalence of an hrHPV type in >BMD could be attributed to the presence of an underlying histological cofounders ≥CIN2 lesion. The >BMD group was split into two groups using ≥CIN2 as threshold. Separate ORs were calculated for ≥CIN3, being the immediate precursor lesion of cervical cancer.

We repeated the analyses for women with a double or ≥triple hrHPV infection. Because sample sizes per age stratum were small and age was not associated with cytological grade for women with multiple infections, strata were pooled. Statistical significance of the ORs was assessed by Fisher-exact tests. For each hrHPV type, we also assessed whether the presence of particular combinations was associated with ≥CIN2 (Fisher-exact test). Finally, we selected all women that were positive for a certain hrHPV type and assessed whether the prevalence of ≥CIN2 was associated with single or multiple infections by OR.

A limitation of any cross-sectional observational study is that ORs may be affected by bias. By using only data from 1 population-based cohort and by controlling for age, we ruled out overt bias. To strengthen the interpretation of the ORs as reflecting an elevated risk of ≥CIN2 for certain hrHPV types, we further examined the sensitivity of the significant associations with regard to hidden, uncontrollable, bias. The latter type of bias occurs when the chance of getting infected with a certain hrHPV type differs among participants. To examine whether this possible bias influenced the ORs, the odds of getting infected with a certain hrHPV type for women with normal cytology vs. women who developed ≥CIN2 were changed from 1:1 (no hidden bias) to 1:1.5. The effect of the odds change on Cochran’s Mantel-Haenszel p-value was examined.

Results Study subjects

Of the 2,154 hrHPV positive women, 1,467 had normal cytology, 374 BMD and 313 >BMD. The mean age per cytological
grade was 38.5 (range 29–61) for women with normal cytology, 36.2 (range 29–61) for those with BMD and 36.8 (range 29–60) for women having BMD. Amongst the hrHPV positive women with >BMD, 245 (78.3%) had an underlying ≥CIN2 lesion, comprising 53 CIN2, 183 CIN3, 5 SCCs and 1 adenocarcinoma. The remaining women with >BMD showed no CIN (n = 9; 2.9%), CIN1 (n = 29; 9.2%) or had no available histological follow-up data (n = 30; 9.6%).

Infections with a single hrHPV type were found in 1,749 (81.2%) women. Another 339 (15.7%) women had a double infection and 66 (3.0%) a ≥triple infection. These figures were 1,221 (83.2%), 212 (14.5%) and 31 (2.3%) for women with normal cytology and 193 (78.8%), 40 (16.3%) and 12 (4.9%) for women with ≥CIN2. Except for 1 case having a triple infection, all women with SCC were positive for a single hrHPV type. The adenocarcinoma was positive for a single type.

**hrHPV type-specific prevalence in women with single infections**

Table I shows the hrHPV type distribution for single infections by cytological and histological classification. Women with single hrHPV infections having >BMD were significantly more likely to contain HPV 16 than those with normal cytology (OR 3.18, 95% CI 2.39–4.21; Table I), whereas women with BMD were not (OR 1.26, 95% CI 0.95–1.66). Amongst women with >BMD, only those with a histologically confirmed ≥CIN2 or ≥CIN3 were significantly more likely to contain HPV 16 compared to women with normal cytology (OR 4.10, 95% CI: 2.98–5.64 and 4.35, 95% CI: 3.05–6.20, respectively). Women with single hrHPV positive cervical carcinomas contained either HPV 16 (5 cases; 71.4%) or HPV 18 (3 cases), the latter including the single adenocarcinoma. The prevalence of HPV 16 in women with ≥BMD without a histologically confirmed ≥CIN2 did not differ from that in women with normal cytology (OR 1.25; 95% CI 0.69–2.26).

After exclusion of HPV 16 positive women, HPV 18 and 33 prevalences were increased in >BMD smears (OR 2.09, 95% CI 1.27–3.43 and OR 2.13, 95% CI 1.18–3.84, respectively) but not in BMD. Amongst women with >BMD, the prevalence of HPV 33 showed a significant increase only in those with a histologically confirmed ≥CIN2 and ≥CIN3 (OR 2.68, 95% CI 1.39–5.15 and OR 3.42, 95% CI 1.72–6.82, respectively), whereas HPV 18 was not. Women with ≥CIN2 were also significantly more likely to contain HPV 58 (OR 2.15, 95% CI 1.04–4.43). Conversely, the prevalence of HPV 56, 59 and 66 was decreased in >BMD, but not in BMD. Considering these types in women with ≥BMD or ≥CIN3, only type 66 was less prevalent. Additionally, women with ≥CIN3 were less likely to contain HPV 45 (Table I). Only 1 significant OR was associated with age (type 18, normal vs. >BMD, p = .002). For the other significant ORs, Breslow-Day p-values ranged from .113 to .937.

Under the assumption of substantial hidden bias, associations remained present only for HPV 16 (≥CIN2, p < .0001; ≥CIN3, p < .0001) and for HPV 33 (≥CIN2, p = .077; ≥CIN3, p = .016). Altogether, these analyses show that there is an increased prevalence of type 16 and 33 in women with ≥CIN2.

**hrHPV type-specific prevalence in women with multiple infections**

Separate analyses were performed for women with double and ≥triple infections. In analogy to women with a single hrHPV infection, women with double infections having >BMD showed an increased prevalence HPV 16 compared to women with normal cytology (Table II), but only in case of a histologically confirmed ≥CIN2 and ≥CIN3 (OR 3.29, 95% CI 1.61–6.75 and OR 6.34, 95% CI 2.49–16.17, respectively). Similar as in women with a single infection, an increased prevalence of type 33 was observed in women with ≥CIN2 and ≥CIN3 (OR 4.37, 95% CI 1.17–16.34 and OR 9.38, 95% CI 1.78–54.20, respectively). The presence of ≥CIN2 was not associated with a particular combination of hrHPV types. For ≥triple infections, no significant differences in hrHPV type distribution were found that could be associated with

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**Table I: hrHPV type distribution for single infections.**

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**Table II: Prevalence of types 16 and 33.**

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cytological or histological grade (data not shown). A comparative analysis revealed that the prevalence of ≥CIN2 was not different in women with a single infection or with a multiple infection, neither for HPV 16 nor for HPV 33 (OR 1.01, 95% CI; 0.65–1.57 and OR 1.69, 95% CI; 0.52–5.47, respectively) The increased prevalences remained detectable when assuming hidden bias both for HPV 16 (≥CIN2, p = .029; ≥CIN3, p = .001) and for HPV 33 (≥CIN2, p = .10; ≥CIN3, p = .016). In women with double infections, the prevalences of types 16 and 33 were increased in case of underlying ≥CIN2. No evidence was found that the increased prevalences were influenced by coexisting hrHPV types.

Discussion

Our comparative study of the distribution of 14 hrHPV types in a population-based screening cohort revealed remarkable differences between the cytological classes. Compared to women with single infections having normal cytology, those with >BMD had a significantly different prevalence of 6 of the hrHPV types analyzed. Amongst these, HPVs 16, 18 and 33 were overrepresented and HPV6s 56, 59 and 66 underrepresented or absent in >BMD women. Only for HPV 16 and HPV 33, the prevalence was elevated both in women with ≥CIN2 and those with ≥CIN3, whereas only for HPV 66 the prevalence was decreased. Our data are suggestive of an increased potential of HPV types 16 and 33 to trigger progression to high-grade CIN disease. Although HPV 18 was overrepresented in women with >BMD, this was not the case when considering only the >BMD women with ≥CIN2.

Analyses for women with double infections revealed the same results as for women with single infections, which supports the conclusion that HPV 16 and 33 are overrepresented in case of ≥CIN2 and ≥CIN3. The increased prevalences of these 2 types in women with ≥high-grade CIN were not influenced by any specific coexisting type, nor was the prevalence of ≥CIN2 in HPV 16 or 33 positive women influenced by the number of co-existing HPVs. This suggests that in multiple infections only a single hrHPV type is responsible for the progression to lesions ≥CIN2 and that there is no synergistic effect of coexisting hrHPV types.

For our study, we considered women with normal cytology as having no underlying ≥CIN2 lesion since in the setting of the current practice standards in nationwide screening, women with normal cytology are considered to be free of disease for a period of 5 years and are not referred for colposcopic evaluation. It should be realized however that this group of hrHPV positive women with normal cytology may include a small proportion of women in whom either an underlying high-grade lesion is missed or will develop during follow-up. When these women would have been recognized and excluded from this group, odds ratios of HPV 16 and HPV 33 for ≥CIN2 would even be higher.

A possible explanation for the increased prevalence of hrHPV type 16 and to a lesser extent HPV 33 in women with ≥CIN2 could be an increased persistence frequency compared to other hrHPV types, either resulting or not from a higher degree of immune escape related to these types. Indeed, in a recent analysis of the natural history of HPV infections in female university students, it appeared that HPV 16 was the most persistent type. On the other hand, these types could also more easily undergo a switch from a productive infection state towards a state indicative of virus-induced transformation as a result of deregulated expression of the viral oncoproteins E6 and E7. The latter phenomenon particularly becomes manifest in CIN2/CIN3 lesions and is suggested to be reflected by an increased expression of the p16INK4a cyclin-dependent kinase inhibitor in the dysplastic cell layers. According to this concept many infections of the remaining hrHPV types, in particular of HPV4s 45, 59, 66 and 68, would at maximum end up in a low-grade CIN lesion, reflecting a productive infection with a relatively low likelihood of undergoing a switch towards virus-induced transformation.

Except for HPV 16, the hrHPV types that in our study showed an increased prevalence in ≥CIN2 lesions (i.e., HPV 16 and HPV 33) are different from those found by Clifford et al. The latter phenomenon might be that the risk posed by HPV 33 for progression to high-grade CIN is relatively high but for the subsequent progression to invasive carcinoma relatively low. Whether this is owing to a lower oncogenic potential of the HPV 33 E6/E7 genes compared to those of HPV 16, 18 and 45, remains to be examined. For HPV 18 and 45, the opposite may be the case, i.e., a relatively lower risk for progression to CIN3, but on the other hand, an increased risk for progression from CIN3 to cervical cancer. This is supported by the observation that HPV 18 infections were relatively less frequent in women, among those with >BMD, who had ≥CIN2, but on the other hand, HPV 18 was, next to HPV 16, the only type that was detected in the series of 8 carcinomas with single infections in this study. Also our recent typing data on a larger series of carcinomas (data not shown) indicate a relatively high prevalence of HPV 18, which is fully in favor of this idea and in line with findings of others. Alternatively, it cannot be excluded that HPV types like HPV 18 and HPV 45 mediate progression to carcinoma within a time interval much shorter than for HPV 16, explaining their relative under representation in high-grade CIN lesions.

That particularly HPV 16 seems to exhibit a different biological behavior compared to other hrHPVs is supported by studies performed on hrHPV containing SCCs of other anatomical sites, such as oral and oropharyngeal carcinomas. HPV 16 accounts for 95% or more of the hrHPV positive tumors in the oral cavity and oropharynx. Our data on the distribution of hrHPV types may have implications for hrHPV test cocktails, not only for cervical screening but also for the triage policy of women with BMD or normal cytology. By distinguishing the hrHPV types with an increased progression risk to CIN3/cervical carcinoma, the specificity of hrHPV testing could be increased, resulting in follow-up algorithms with different time intervals for certain hrHPV types, which may improve the screening efficiency for CIN3 and cervical cancer and consequently the cost-effectiveness. Moreover, our findings may have consequences for strategies on the development of HPV-specific vaccines for the prevention of SCC. Since protection to hrHPV infection is type-specific, prophylactic vaccines that are currently being developed need to be polyvalent and should be tailored particularly to those hrHPV types exhibiting the highest risk of cervical cancer.

In conclusion, we have shown based on cross-sectional data that HPV 16 and HPV 33 were more prevalent in hrHPV positive women with ≥CIN2 and ≥CIN3 than could be expected by their prevalence in women with normal cytology. This suggests that women with normal cytology harboring HPV 16 and 33 have an increased progression rate to ≥CIN2. These results should be confirmed in longitudinal follow-up studies, which are presently under investigation by our group. Our data may result in different HPV type-dependent follow-up algorithms in the future.

Acknowledgements

We gratefully acknowledge the work of the 242 GPs and their assistants, the District Health Authority Amstelveen, Medial, and DHV Kennemerland-Haarlemmermeer e.o., A.J.C. van den Brule, S. Bulk, R. Rozendaal and G. van Zandwijken for their work and support in this study. We especially thank the people of the Unit Molecular Pathology for their technical work in HPV typing.

Contributing members of the POBASCAM study group, other than authors:

Chapter 5 Prevalence of types 16 and 33

PREVALENCE OF TYPES 16 AND 33

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References

Chapter 6

HPV type-specific 18-month risk of high-grade cervical intraepithelial neoplasia in women with a normal or borderline/mildly dyskaryotic smear

Johannes Berkhof, Nicole W.J. Bulkmans, Maaike C.G. Bleeker, Saskia Bulk, Peter J.F. Snijders, Feja J. Voorhorst, and Chris J.L.M. Meijer

Cancer Epidemiology, Biomarkers & Prevention 2006; 15(7): 1268-1273
Human Papillomavirus Type–Specific 18-Month Risk of High-Grade Cervical Intraepithelial Neoplasia in Women with a Normal or Borderline/Mildly Dyskaryotic Smear

Johannes Berkhof,¹ Nicole W.J. Bulkmans,² Maaike C.G. Bleeker,² Saskia Bulk,² Peter J.F. Snijders,² Feja J. Voorhorst,¹ and Chris J.L.M. Meijer²

Departments of ¹Clinical Epidemiology and Biostatistics and ²Pathology, Vrije University Medical Center, Amsterdam, the Netherlands

Abstract

Introduction: High-risk human papillomavirus (hrHPV) DNA testing is an increasingly used instrument in cervical cancer prevention along cervical cytology. The inclusion of hrHPV testing in cervical screening requires efficient management as many hrHPV infections are transient. We investigated the potential value of hrHPV genotyping in normal and borderline/mildly dyskaryotic (BMD) smears.

Materials and Methods: From a screening population of 44,102 women in the Netherlands, we included hrHPV-positive women with a normal or BMD smear. We assessed the type-specific 18-month risk of high-grade cervical intraepithelial neoplasia (CIN).

Results: In hrHPV-positive women, 18-month risk of CIN grade 3 or invasive cancer (≥CIN3) was 6% [95% confidence interval 95% CI, 4-9] after normal cytology and 20% (95% CI, 16-25) after BMD. If positive for HPV16, ≥CIN3 risks were 14% (95% CI, 9-21) and 37% (95% CI, 28-48), respectively. In the subset of hrHPV-positive women without HPV16, HPV18 found in other hrHPV-positive women without HPV16 and HPV18 had an increased risk of high-grade CIN after normal cytology and HPV31 and HPV33 were associated with an increased risk, particularly after BMD. HPV16 and HPV18 were also associated with a higher risk of high-grade CIN in women with an hrHPV-positive normal baseline smear and a repeat normal smear at 6 months.

Discussion: HRHPV-positive women without type 16, 18, and 31 had a relatively lower risk of high-grade CIN. Among women with baseline normal cytology and among women with a baseline and repeat normal smear, HPV16/18-positive women showed an increased risk of high-grade CIN. This warrants more aggressive management of HPV16/18–positive women compared with other hrHPV-positive women. (Cancer Epidemiol Biomarkers Prev 2006;15(7):1268–73)

Novelty and Impact of the Article

This is the first large population-based screening trial in which high-grade cervical intraepithelial neoplasia (CIN) risk for 14 oncogenic HPV types has been determined for all hrHPV-positive baseline smears. Four HPV types could be distinguished that were associated with increased high-grade CIN risk (i.e., HPV16, HPV18, HPV31, and HPV33). In the subset of women with normal cytology at baseline, HPV16- and HPV18-positive women had an increased risk of high-grade CIN compared with women positive for other hrHPV types also after multiple normal smears. Besides, cytology was less predictive for risk of high-grade CIN in HPV16/18–positive women than in other hrHPV-positive women. This seems to warrant more aggressive management for HPV16/18–positive women compared with non-HV16/18 hrHPV-positive women, particularly if the cytologic reading is normal and therefore may have important implications for cervical screening management.

Received 10/4/05; revised 4/7/06; accepted 5/16/06.

Grant support: Zorg Onderzoek Nederland (Netherlands Organisation for Health Research and Development) grant 30-05220. The funding source had no involvement in study design, data collection, analysis and interpretation of the data, writing of the report, and in the decision to submit the paper for publication.

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Contributions: J. Berkhof analyzed the data and prepared the manuscript. N.W.J. Bulkmans was responsible for recruitment of the data and data systems. M.C.G. Bleeker contributed to the design of the study and the data analysis. P.J.F. Snijders was responsible for high-risk HPV testing. C.J.L.M. Meijer was the principal investigator and supervisor of this project. All authors participated in the preparation of the design of the study, interpretation of the data, writing of the manuscript, and approved the final version.

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Introduction

Persistent infection with high-risk human papillomavirus (hrHPV) is the central cause of cervical cancer (1) and several studies have shown that the detection rate of early cancer or clinically relevant precursor lesions can be enhanced by hrHPV DNA testing along cervical cytology (2-6). However, only few hrHPV infections progress to cancer or its closest precursor CIN grade 3 (7).

Distinguishing hrHPV-positive women with an elevated risk of CIN grade 3 or worse (≥CIN3) may result in a better management of hrHPV-positive women. At least to a certain extent, this may be accomplished by an hrHPV type analysis as recent meta-studies have shown that only a few HPV types, including HPV16 and HPV18, cover the vast majority of invasive cancer cases (8, 9). Moreover, the risk of high-grade CIN may vary across types. In a recent cross-sectional study, elevated prevalences of HPV16 and HPV33 were found in moderately dyskaryotic or worse smears with underlying ≥CIN3 compared with normal smears (10) and an increased risk of ≥CIN3 posed by HPV16 was found in a cohort with equivocal or mildly abnormal cytology at enrollment (11). Besides, by HPV16 and HPV18 typing of enrollment smears in a routine screening cohort study, increased risks were found for those two types (12). Together, these study results suggest that the risk posed by different hrHPV types for ≥CIN3 varies across cytologic categories.

The aim of this study was to assess the hrHPV type–specific 18-month risk of high-grade CIN in women with normal cytology and women with borderline/mild dyskaryosis (BMD) participating in a population-based screening program (13). Here, particular attention was paid to the role of baseline cytology. All women were typed for 14 hrHPV genotypes (9).
Materials and Methods

Study Population and Data Collection. From January 1999 to December 2002, 44,102 women of ages between 30 and 60 years and eligible for cervical screening were recruited to participate in the Population-Based Screening Amsterdam trial (13). In this two-armed trial, effectiveness of cytology and hrHPV DNA testing (intervention group) was compared with the effectiveness of cytology (control group, hrHPV DNA results blinded). Written informed consent was provided by all women and the study was approved by the Medical Ethics Committee of the Vrije University Medical Centre (no. 96/103A) and the Ministry of Public Health (VWS no. 328650).

Women in the intervention group (n = 21,996) were referred for immediate colposcopy when the cytologic result was moderate dyskaryosis or worse (>BMD; high-grade squamous intraepithelial lesion according to Bethesda 2001), were returned to the next screening round when the smear result was normal and the hrHPV DNA test result was negative, and were followed with cytologic and hrHPV DNA testing at 6 and 18 months when the smear result was borderline or mild dyskaryosis (BMD; translates into Bethesda 2001 atypical squamous cells of undetermined significance/high-grade squamous intraepithelial lesion/low-grade squamous intraepithelial lesion; ref. 14) or when the smear result was normal and the hrHPV DNA result test was positive. Women with follow-up cytologic and hrHPV DNA testing were referred for colposcopy when the 6-month smear result was >BMD, when the baseline and 6-month smear result was BMD and the 6-month smear was hrHPV positive, or when the 18-month smear was hrHPV positive and/or interpreted as >BMD. Women in the control group (n = 22,106) followed the current Dutch screening guidelines; they were referred for immediate colposcopy when the smear result was >BMD, were returned to the next screening round at 5 years when the result was normal, and were followed with cytology at 6 or 18 months when the result was BMD. Women with follow-up cytology were referred for colposcopy when the 6- or 18-month result was ≥BMD. All women in the control and intervention group were tested for hrHPV at baseline. A flow chart of the screening management of women who were advised to return for repeat testing is presented in Fig. 1.

Conventional cytologic testing was done on smears taken with Cervex brush. Detection of hrHPV DNA in the scrapes was done by GP5+/6+ PCR enzyme immunoassay using a cocktail of 14 high-risk types (i.e., 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68; ref. 15). GP5+/6+ PCR-positive cases were subsequently typed by reverse line blotting (16).

Statistical Analysis. From the women enrolled in the Population-Based Screening Amsterdam study, we included all hrHPV-positive women with normal cytology from the intervention group (n = 763) and with BMD from the intervention (n = 185) and control (n = 196) groups. Women negative on reverse line blot typing (n = 57) were excluded, leaving 713 hrHPV-positive women with normal cytology and 374 hrHPV-positive women with BMD for the analyses. We calculated 18-month type-specific cumulative absolute risks of CIN grade 2 or worse (≥CIN2) and ≥CIN3. Kaplan-Meier estimates were used and 95% confidence intervals (95% CI) for the cumulative risk were obtained by bootstrapping (17). If all women positive for a certain HPV type were censored (i.e., no cases of ≥CIN2 or ≥CIN3) or the sample size was very small (<10), a binomial reference distribution was assumed for the estimated risk. Time was set equal to the target referral date (6 or 18 months), reasoning that high-grade lesions were already present at the time of referral. CIN cases that were not histologically confirmed within 3 years after the baseline smear (13 of 179) were censored as they might have been developed after the referral date. Censoring was also applied to women who were lost to follow-up. Preferential risk of ≥CIN2 and ≥CIN3 was tested for each HPV type separately using the exact stratified log-rank test (18). Data were stratified in three age categories corresponding to the first round in nationwide screening, the second round, and rounds 3 to 7 (i.e., 29-33, 34-38, and 39-63 years). Exact P values were obtained via

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**Figure 1.** Management of women in the Population-Based Screening Amsterdam study who were advised to return for repeat testing at 6 and 18 months.
HPV Type-Specific Risk of High-Grade CIN

Table 1. hrHPV type distribution and cumulative 18-month risks of ≥CIN2 and ≥CIN3 in women with an hrHPV-positive normal smear

<table>
<thead>
<tr>
<th>hrHPV type at baseline</th>
<th>≥CIN2</th>
<th>≥CIN3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Risk, % (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Single + multiple hrHPV infections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>13 (10-17)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>16</td>
<td>27 (20-35)</td>
<td></td>
</tr>
<tr>
<td>HPV16-positive cases excluded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>7 (5-11)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>18</td>
<td>18 (7-35)</td>
<td>0.022</td>
</tr>
<tr>
<td>31</td>
<td>10 (4-20)</td>
<td>0.384</td>
</tr>
<tr>
<td>33</td>
<td>20 (6-44)</td>
<td>0.027</td>
</tr>
<tr>
<td>35</td>
<td>4 (0-23)</td>
<td>0.828</td>
</tr>
<tr>
<td>39</td>
<td>0 (0-11)</td>
<td>0.143</td>
</tr>
<tr>
<td>45</td>
<td>0 (0-6)</td>
<td>0.063</td>
</tr>
<tr>
<td>51</td>
<td>8 (0-27)</td>
<td>0.767</td>
</tr>
<tr>
<td>52</td>
<td>15 (4-34)</td>
<td>0.068</td>
</tr>
<tr>
<td>56</td>
<td>0 (0-6)</td>
<td>0.081</td>
</tr>
<tr>
<td>58</td>
<td>0 (0-8)</td>
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</tr>
<tr>
<td>59</td>
<td>0 (0-18)</td>
<td>0.446</td>
</tr>
<tr>
<td>66</td>
<td>2 (0-13)</td>
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<tr>
<td>68</td>
<td>0 (0-26)</td>
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</tr>
<tr>
<td>Single hrHPV infections only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>14 (11-18)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>16</td>
<td>31 (22-41)</td>
<td></td>
</tr>
<tr>
<td>HPV16-positive cases excluded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>8 (5-12)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>18</td>
<td>18 (5-41)</td>
<td>0.077</td>
</tr>
<tr>
<td>31</td>
<td>11 (4-23)</td>
<td>0.341</td>
</tr>
<tr>
<td>33</td>
<td>25 (8-54)</td>
<td>0.022</td>
</tr>
<tr>
<td>35</td>
<td>6 (0-33)</td>
<td>0.972</td>
</tr>
<tr>
<td>39</td>
<td>0 (0-17)</td>
<td>0.169</td>
</tr>
<tr>
<td>45</td>
<td>0 (0-8)</td>
<td>0.113</td>
</tr>
<tr>
<td>51</td>
<td>13 (0-44)</td>
<td>0.402</td>
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<td>52</td>
<td>14 (4-38)</td>
<td>0.131</td>
</tr>
<tr>
<td>56</td>
<td>0 (0-8)</td>
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<td>0 (0-11)</td>
<td>0.108</td>
</tr>
<tr>
<td>59</td>
<td>0 (0-28)</td>
<td>0.554</td>
</tr>
<tr>
<td>66</td>
<td>3 (0-19)</td>
<td>0.491</td>
</tr>
<tr>
<td>68</td>
<td>0 (0-52)</td>
<td>0.461</td>
</tr>
</tbody>
</table>

Abbreviations: ACIS, adenocarcinoma in situ; SCC, squamous cell carcinoma; AdCa, adenocarcinoma.

Results

Study Subjects. The mean age of hrHPV-positive women with normal cytology at baseline was 38.3 years (range, 29-60 years) and the mean age of hrHPV-positive women with BMD at baseline was 36.2 years (range, 29-59 years). In women of the intervention group with normal cytology, 23.1% (165 of 713) did not respond to a follow-up invitation at 6 months and 28.0% (146 of 522) did not respond to a second follow-up invitation at 18 months. Among the responders, 1 (0.1%) squamous cell carcinoma, 1 (0.1%) adenocarcinoma \textit{in situ}, 27 (3.8%) CIN3 cases, and 29 (4.1%) CIN2 cases were detected during follow-up. For women with BMD, the nonresponse rates at follow-up invitations at 6 and 18 months were 9.9% (37 of 374) and 28.8% (53 of 184), respectively. In women of the intervention group with BMD, 1 (0.6%) adenocarcinoma, 2 (1.1%) squamous cell carcinoma, 1 (0.6%) adenocarcinoma \textit{in situ}, 29 (16.0%) CIN3 cases, and 21 (11.6%) CIN2 cases were detected during follow-up, and in women of the control group with BMD, 1 (0.5%) adenocarcinoma, 1 (0.5%) squamous cell carcinoma, 28 (14.5%) CIN3 cases, and 24 (12.4%) CIN2 cases were detected.

Multiple hrHPV infections were less prevalent in women with normal cytology than in women with BMD (18.0% versus 24.6%; P = 0.011, Fisher’s exact test). The women with invasive cancer (n = 6) were positive for only one hrHPV type; four contained HPV16, one HPV18, and another one HPV31. Loss to follow-up was not type specific (P > 0.05 for each type, Fisher’s exact test).

Cumulative Absolute ≥CIN2 and ≥CIN3 Risk in Women with Normal Cytology. The cumulative 18-month ≥CIN2 and ≥CIN3 risks are presented in Table 1. Overall risks were 13% (95% CI, 10-17) and 6% (95% CI, 4-9), respectively. HPV16-positive women had a ≥CIN2 risk of 27% (95% CI, 20-35) and a ≥CIN3 risk of 14% (95% CI, 9-21). In the subset of hrHPV-positive women without HPV16, overall risks of ≥CIN2 and ≥CIN3 were 7% (95% CI, 5-11) and 3% (95% CI, 1-6). HPV18-positive women had significantly elevated ≥CIN2 (18%; 95% CI, 7-35) and ≥CIN3 (9%; 95% CI, 2-23) risks compared with HPV18-negative women. HPV31-positive women had a significantly elevated ≥CIN3 risk of 7% (95% CI, 2-16) compared with HPV31-negative women and had a significantly elevated ≥CIN2 risk of 20% (95% CI, 6-44). Risk estimates remained similar when excluding women with multiple infections.

Cumulative Absolute ≥CIN2 and ≥CIN3 Risk in Women with BMD. The cumulative 18-month risks of high-grade CIN in women with BMD are presented in Table 2. Overall risks of simulation. Because HPV16 is considered the most prevalent HPV type, risk estimates and log-rank tests for non-HPV16 types were calculated after discarding HPV16-positive cases. To examine the effect of coexisting types, analyses were repeated for single infections only. Type-specific relative risks of baseline cytology (BMD versus normal) on ≥CIN2 and ≥CIN3 risk were estimated by Cox regression. The effects of age cohort on ≥CIN2 and ≥CIN3 risk were tested by the log-rank test. Calculations were done with SPSS9.0 and Matlab7.0.
\( \geq \text{CIN2} \) and \( \geq \text{CIN3} \) were 34% (95% CI, 29-39) and 20% (95% CI, 16-25). In HPV16-positive women, \( \geq \text{CIN2} \) risk was 48% (95% CI, 38-58) and \( \geq \text{CIN3} \) risk was 37% (95% CI, 28-48). In the subset of hrHPV-positive women without HPV16, overall \( \geq \text{CIN2} \) and \( \geq \text{CIN3} \) risks were 27% (95% CI, 21-33) and 12% (95% CI, 8-17). HPV31-positive women had an elevated \( \geq \text{CIN3} \) risk of 27% (95% CI, 14-46) compared with HPV31-negative women and had elevated \( \geq \text{CIN2} \) and \( \geq \text{CIN3} \) risks of 49% (95% CI, 29-71) and 22% (95% CI, 9-44). Different from the results for women with normal cytology at baseline, HPV18 was not associated with an elevated risk of \( \geq \text{CIN2} \) or \( \geq \text{CIN3} \). Risk estimates remained similar when excluding women with multiple infections.

**Cumulative Absolute \( \geq \text{CIN2} \) and \( \geq \text{CIN3} \) Risk in Women with Multiple Normal Smears.** Of all women with \( \geq \text{CIN2} \) and normal cytology at baseline \( (n = 58) \), 14 (24%) women also had normal cytology at the first recall at 6 months and 9 (16%) women had normal cytology at both 6 and 18 months. These figures indicate that women with an hrHPV infection at baseline may have a substantial risk of high-grade CIN even when the baseline smear is followed by one or two normal smears. In subsequent analyses, we assessed whether the risk of high-grade CIN after multiple normal smears was associated with HPV types 16 and 18. Cumulative 18-month risk estimates of \( \geq \text{CIN2} \) and \( \geq \text{CIN3} \) in women with an hrHPV-positive normal smear at baseline and a normal repeat smear at 6 months are presented in Table 3.

Overall 18-month risk estimates of \( \geq \text{CIN2} \) and \( \geq \text{CIN3} \) for this group of hrHPV-positive women were 6% (95% CI, 4-10) and 2% (95% CI, 1-4). Women with HPV16 at baseline had an elevated \( \geq \text{CIN2} \) risk of 13% (95% CI, 7-24; \( P = 0.004 \)) compared with hrHPV-positive women without HPV16. Presence of HPV16 was not associated with an elevated \( \geq \text{CIN3} \) risk \( (P = 0.548) \). When positive for HPV18, and after exclusion of those positive for HPV16, \( \geq \text{CIN2} \) and \( \geq \text{CIN3} \) risks were 14% (95% CI, 5-37; \( P = 0.007 \)) and 5% (95% CI, 1-28; \( P = 0.037 \)), indicating an increased risk for HPV18 as well. Inspection of the hrHPV status at 6 months revealed that all four CIN3 cases were detected after two hrHPV-positive normal smears and 1 of 10 CIN2 cases was hrHPV-negative at 6 months. The latter case was HPV18 positive at baseline. In case the baseline smear was positive for hrHPV types other than types 16 and 18 and the 6-month smear was still hrHPV positive, 18-month \( \geq \text{CIN2} \) and \( \geq \text{CIN3} \) risks were only 2% (95% CI, 1-9) and 1% (95% CI 0-8).

In women with an hrHPV-positive normal smear at baseline and two subsequent normal smears at 6 and 18 months, \( \geq \text{CIN2} \) and \( \geq \text{CIN3} \) rates were 4% (95% CI, 2-8) and 1% (95% CI, 0-3). \( \geq \text{CIN2} \) rates were significantly different \( (P = 0.028) \) in women with baseline HPV16 and/or HPV18 (9%; 95% CI, 4-18) and women positive for another hrHPV type at baseline (2%; 95% CI, 0-6).

**Effect of Baseline Cytology and Age Cohort.** The relative risk of \( \geq \text{CIN2} \) in women with a baseline hrHPV-positive BMD smear compared with those with an hrHPV-positive normal smear was 2.17 (95% CI, 1.40-3.37) if positive for HPV16, 1.45 (95% CI, 0.54-3.90) if positive for HPV18, and 5.22 (95% CI, 2.94-9.26) if positive for another hrHPV type. The difference between the relative risks in HPV16/18-positive women and in women positive for another hrHPV type was statistically

Table 2. hrHPV type distribution and cumulative 18-month risks of \( \geq \text{CIN2} \) and \( \geq \text{CIN3} \) in women with an hrHPV-positive BMD smear

<table>
<thead>
<tr>
<th>hrHPV type at baseline</th>
<th>( \geq \text{CIN2} )</th>
<th>( \geq \text{CIN3} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Risk, % (95% CI)</td>
<td>( P )</td>
</tr>
<tr>
<td>Single + multiple hrHPV infections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>34 (29-39)</td>
<td>0.0006</td>
</tr>
<tr>
<td>16</td>
<td>37 (38-58)</td>
<td></td>
</tr>
<tr>
<td>HPV16-positive cases excluded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>27 (21-33)</td>
<td>0.016</td>
</tr>
<tr>
<td>18</td>
<td>29 (15-47)</td>
<td>0.883</td>
</tr>
<tr>
<td>31</td>
<td>37 (23-54)</td>
<td>0.016</td>
</tr>
<tr>
<td>33</td>
<td>49 (29-71)</td>
<td>0.001</td>
</tr>
<tr>
<td>35</td>
<td>10 (3-33)</td>
<td>0.004</td>
</tr>
<tr>
<td>39</td>
<td>19 (7-39)</td>
<td>0.842</td>
</tr>
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<td>0.229</td>
</tr>
<tr>
<td>51</td>
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</tr>
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<td>25 (10-50)</td>
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</tr>
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<td>15 (7-36)</td>
<td>0.507</td>
</tr>
<tr>
<td>58</td>
<td>34 (16-61)</td>
<td>0.122</td>
</tr>
<tr>
<td>59</td>
<td>50 (0-81)</td>
<td>0.115</td>
</tr>
<tr>
<td>66</td>
<td>13 (0-41)</td>
<td>0.526</td>
</tr>
<tr>
<td>68</td>
<td>36 (3-60)</td>
<td>0.545</td>
</tr>
<tr>
<td>Single hrHPV infections only</td>
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<td></td>
</tr>
<tr>
<td>Any</td>
<td>34 (28-40)</td>
<td>0.009</td>
</tr>
<tr>
<td>16</td>
<td>46 (35-58)</td>
<td></td>
</tr>
<tr>
<td>HPV16-positive cases excluded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>28 (21-36)</td>
<td>0.0001</td>
</tr>
<tr>
<td>18</td>
<td>43 (20-73)</td>
<td>0.871</td>
</tr>
<tr>
<td>31</td>
<td>44 (26-64)</td>
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</tr>
<tr>
<td>33</td>
<td>50 (23-76)</td>
<td>0.026</td>
</tr>
<tr>
<td>35</td>
<td>20 (10-70)</td>
<td>0.739</td>
</tr>
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<td>39</td>
<td>10 (0-57)</td>
<td>0.940</td>
</tr>
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<td>45</td>
<td>13 (0-47)</td>
<td>0.481</td>
</tr>
<tr>
<td>51</td>
<td>24 (10-50)</td>
<td>0.340</td>
</tr>
<tr>
<td>52</td>
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<td>58</td>
<td>46 (17-80)</td>
<td>0.265</td>
</tr>
<tr>
<td>59</td>
<td>0 (0-84)</td>
<td>0.655</td>
</tr>
<tr>
<td>66</td>
<td>0 (0-31)</td>
<td>0.346</td>
</tr>
<tr>
<td>68</td>
<td>33 (0-58)</td>
<td>0.590</td>
</tr>
</tbody>
</table>
Our results showed that HPV16-positive women with normal cytology and BMD had strongly increased 18-month absolute risks of high-grade CIN compared with hrHPV-positive women who were negative for HPV16. In the latter group, increased risks of high-grade CIN were found for HPV16 and/or HPV18 for the majority of invasive cervical cancers (8, 21), further endocervical inspection including for instance endocervical curetage may be justified for HPV16/18–positive women when the transformation zone is found normal by colposcopy (22).

Some methodologic issues need to be discussed. First, women belonging to the three categories (i.e., intervention group-normal cytology, intervention group-BMD, and control group-BMD) were subjected to slightly different referral strategies. However, it is unlikely that this led to detection bias in the intervention group as women in that group only returned to routine screening when the hrHPV infection cleared and the cytologic result was normal or BMD. For women in the control group with BMD, management was not based on hrHPV DNA because the result of the hrHPV test was blinded during follow-up and cytologically poorly accessible lesions might have been missed. However, distribution of histologically diagnosed lesions in women with BMD of the control and intervention group were very similar.

**Table 3. Baseline hrHPV type distribution and cumulative 18-month risks of ≥CIN2 and ≥CIN3 in women with an hrHPV-positive smear at baseline and a normal repeat smear at 6 months**

<table>
<thead>
<tr>
<th>hrHPV type at baseline</th>
<th>At risk CIN2</th>
<th>CIN3</th>
<th>≥CIN2</th>
<th>P</th>
<th>≥CIN3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td></td>
<td>Risk (95% CI)</td>
<td></td>
<td>Risk (95% CI)</td>
</tr>
<tr>
<td>Irrespective of hrHPV status at 6 mo</td>
<td></td>
<td></td>
<td>Risk, %</td>
<td></td>
<td>Risk, %</td>
</tr>
<tr>
<td>Any</td>
<td>373</td>
<td>10</td>
<td>6 (4-10)</td>
<td></td>
<td>2 (1-4)</td>
</tr>
<tr>
<td>16</td>
<td>104</td>
<td>7</td>
<td>13 (7-24)</td>
<td>0.004</td>
<td>3 (1-11)</td>
</tr>
<tr>
<td>16 and/or 18</td>
<td>135</td>
<td>3</td>
<td>13 (8-22)</td>
<td>0.0004</td>
<td>3 (1-10)</td>
</tr>
<tr>
<td>18 after exclusion 16</td>
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<td>5 (1-28)</td>
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<td></td>
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<td>1 (0-8)</td>
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<tr>
<td>hrHPV-negative at 6 mo</td>
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<td>No 16, 18</td>
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<td>0</td>
<td>0 (0-4)</td>
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<td>0 (0-4)</td>
</tr>
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</table>
thus, it is not likely that results were substantially affected by the referral strategies. Second, 23% of the women with normal cytology and 10% of the women with BMD were lost to follow-up at 6 months and nearly 30% of the women did not show up at the second invitation at 18 months. We accounted for loss to follow-up by applying Kaplan-Meier censored analyses. This may invalidate the results when censoring is HPV type specific, which cannot be excluded beforehand. However, we did not find an association between HPV type and censoring. Third, HPV types 59 and 68 were relatively rare; thus, it would have been difficult to find an increased risk of high-grade CIN for any of these two types. However, the four types which were significantly associated with risk of high-grade CIN also had a relatively high absolute ≥CIN2 and ≥CIN3 risk and therefore were not merely singled out because they were the most common ones.

An important strength of our prospective trial study of 44,102 women is that the included women had age ≥30 years and were eligible for screening so that the effectiveness of HPV typing in current screening can be directly assessed. In our study, the prevalence of HPV16- and HPV18-positive normal smears was 37% (262 of 713) and the prevalence of HPV16-, HPV31-, and HPV33-positive BMD smears was 52% (195 of 374). Hence, both after normal cytology and after BMD, HPV typing can be used to identify subgroups of hrHPV-positive women for which different follow-up algorithms may lead to an improvement in screening management.

In conclusion, in hrHPV-positive women, we identified four hrHPV types that were associated with a substantially increased risk of high-grade CIN. Adjunct hrHPV typing therefore enables distinguishing risk classes for high-grade CIN and may lead to considerable improvements in screening management. Identification of HPV16 or HPV18 is of utmost importance because women with a persistent infection with one of these types are at risk of prevalence of incipient high-grade CIN also when these are not detected by cytology.

Appendix A. Population-Based Screening Amsterdam Study Collaborators Other than Authors

K. van Groningen (Spaarne Ziekenhuis, Heemstede), W. Ruizinga (Stichting PA Laboratorium Kennemerland, Haarlem), M.E. Boon (Leiden Cytology and Pathology Laboratory, Leiden), M. van Ballegooijen (Department of Public Health and Social Medicine, Erasmus University Rotterdam), A.J.P. Boeke (Institute for Research in Extramural Medicine, Vrije University Medical Centre, Amsterdam), Prof. R.H.M. Verheijen (Department of Obstetrics and Gynaecology, Vrije University Medical Centre, Amsterdam), and F. van Kemende and L. Rozendaal (Unit cytopathology, Vrije University Medical Centre, Amsterdam).

Acknowledgments

We thank the 242 GPs and their assistants, the District Health Authority Amstelveen, Medial, and DHV Kennemerland-Haarlemmermeer e.o.; the research analysts of the Unit Molecular Pathology, Vrije University Medical Centre, Amsterdam for hrHPV testing and HPV typing; and the cytotecnologists (Spaarne Ziekenhuis, Heemstede; Stichting PA Laboratorium Kennemerland, Haarlem; Leiden Cytology and Pathology Laboratory, Leiden; Unit Cytopathology, Vrije University Medical Centre, Amsterdam) for cytologic testing.

References

Risk of high-grade cervical intra-epithelial neoplasia based on cytology and high-risk HPV testing at baseline and at 6-months

Saskia Bulk, Nicole W.J. Bulkmans, Johannes Berkhof, Lawrence Rozendaal, A. Joan P. Boeke,
René H.M. Verheijen, Peter J.F. Snijders, and Chris J.L.M. Meijer

Risk of high-grade cervical intra-epithelial neoplasia based on cytology and high-risk HPV testing at baseline and at 6-months

Saskia Bulk1, Nicole W.J. Bultkams2, Johannes Berkhof3, Lawrence Rozendaal1, A. Joan P. Boeke3, Rene H.M. Verheijen4, Peter J.F. Snijders5 and Chris J.L.M. Meijer6,8

1Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands
2Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, VU University Medical Center, The Netherlands
3Department of Clinical Epidemiology & Biostatistics, VU University Medical Center, Amsterdam, The Netherlands
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5Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands

Adding a test for high-risk human papillomavirus (hrHPV) to cytological screening enhances the detection of high-grade cervical intraepithelial neoplasia (CIN2), but data are required that enable a long-term evaluation of screening. We investigated the CIN2 risk for women participating in population-based screening as a function of hrHPV and cytology testing results at baseline and at 6 months. We included 2,193 women aged 30–60 years participating in a population-based screening trial who received colposcopy or a repeat testing advice at baseline. The main endpoint was histologically confirmed CIN2 diagnosed within 36 months. hrHPV testing was more sensitive than cytology for CIN2 (relative sensitivity 1.4, 95%CI: 1.3–1.5; absolute sensitivity 94.1 and 68.0%, respectively). The 18-month CIN2 risks in women with a hrHPV-positive smear and in women with abnormal cytology were similar (relative risk 0.9, 95%CI: 0.8–1.1). Women with HPV16 and/or HPV18 had a higher CIN2 risk than other hrHPV-positive women irrespective of the cytological grade. Repeat testing showed that both cytological regression and viral clearance were strongly associated with a decrease in CIN2 risk. Notably, women who had a double negative repeat test at 6 months had a CIN2 risk of only 0.2% (95%CI: 0.0–1.1) and hrHPV-negative women with baseline borderline or mild dyskaryosis and normal cytology at 6 months had a CIN2 risk of 0% (95%CI: 0.0–0.8). Using hrHPV and/or cytology testing, risk of CIN2 can be assessed more accurately by repeat testing than single visit testing. Hence, when hrHPV testing is implemented, patient management with repeat testing is a promising strategy to control the number of referrals for colposcopy.

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Key words: human papillomavirus; screening; cervical cancer; sensitivity

Invasive cervical cancer is one of the leading causes of cancer-related death in women of childbearing age worldwide.1,2 As a preventive measure, screening by cervical cytology (i.e., the Pap test) has been shown to dramatically decrease the cervical cancer incidence and mortality.3 Another possibility is to screen for infections with high-risk human papillomavirus (hrHPV), the causative agent for cervical cancer, and combined cytology and hrHPV testing seems to be a promising strategy to improve cervical screening. Previous studies have shown that cytology combined with hrHPV testing improves the sensitivity to detect high-grade cervical lesions.4–6 The positive predictive value of a single positive hrHPV test, however, remains low for women with a normal smear or mild cytological abnormalities, and referral rates for colposcopy may increase substantially with combined testing.6 Hence, implementation of hrHPV testing needs to be preceded by an evaluation of various screening strategies using hrHPV and cytological testing.

We investigated the risk of high-grade cervical intraepithelial neoplasia in women with hrHPV test results and cytology at baseline and at 6 months. We used data obtained from a population-based cervical screening trial. Since women in whom HPV16 or HPV18 is detected seem to have a substantially elevated risk of high-grade lesions compared with other hrHPV-positive women, and since 70% of all cases of cervical cancer are caused by HPV16 and HPV18, we evaluated risks for hrHPV-positive women with HPV16 and/or HPV18 and women positive for other high-risk types separately.7,8

Material and methods

Study population and procedures

In this study, we included all women participating in the POBASCAM (Population-Based Screening Amsterdam) trial who had received an advice to have repeat cytology at 6 and 18 months, or who had been referred for immediate colposcopy. The POBASCAM trial is a population-based double blind randomized controlled trial to evaluate the efficacy of screening using hrHPV testing in conjunction with conventional cytology (intervention group) compared with cervical screening with classical cytology (control group). All participants gave written informed consent. The design, methods and baseline results of the POBASCAM trial have been described previously.9 A flowchart of the randomization, selection and screening procedure of the POBASCAM trial is presented in Figure 1.

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1Saskia Bulk and Nicole W.J. Bultkams have contributed equally to this manuscript. Bulk performed the analyses and prepared the manuscript. N.W.J. Bultkams was responsible for the data collection. J. Berkhof was responsible for the conception of the study and statistical methods. P.J.F. Snijders was responsible for the virology determinations. C.J.L.M. Meijer was the principal investigator of the project, and had access to all data and final responsibility for the decision to submit for publication. All authors participated in the preparation of the design of the study, interpretation of data, writing of the manuscript and approved the final version of the manuscript.

POBASCAM study collaborators other than authors: Dr. K. V Groening (Spaarne Ziekenhuis, Hoofddorp), Dr. W. Ruitinga (Kennemer Gasthuis, Haarlem), Dr. M.E. Boon (Leiden Cytology and Pathology Laboratory, Leiden), Dr. M. van Ballegooijen (Department of Public Health and Social Medicine, Erasmus University Rotterdam), Dr. F.J. Voorhorst and Dr. F.J. van Kemenade (Unit cytopathology, VU University Medical Centre, Amsterdam).

Abbreviations: BMD, borderline or mild dyskaryosis; >BMD, moderate dyskaryosis or worse; >CIN2, high-grade cervical intraepithelial neoplasia or worse, i.e., CIN2, CIN3 or invasive cancer; >CIN3, high-grade cervical intraepithelial neoplasia or worse, i.e., CIN3 or invasive cancer; HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; POBASCAM, population based screening Amsterdam.

Grant sponsor: ZonMW (Zorg Onderzoek Nederland, Netherlands Organisation for Health Research and Development); Grant numbers: 30–05220, 2200.0089.

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Received 4 September 2006; Accepted after revision 23 January 2007 DOI 10.1002/cncr.22677

Published online 12 March 2007 in Wiley InterScience (www.interscience.wiley.com).
Conventional cytological smears were taken using a Cervex-Brush (Rovers Medical Devices, Oss, The Netherlands). The brush was placed in a vial containing a collection medium (i.e., 5 ml PBS and 0.5% thiomersal) for hrHPV testing. Cervical smears were classified according to the Dutch CISOE-A classification blinded to hrHPV status of participants. In short, cytological

**Figure 1** – N indicates normal cytology, BMD indicates borderline or mild dyskaryosis and >BMD indicates moderate dyskaryosis or worse. Participants in the intervention group (n = 21,996) were referred for colposcopy directed biopsy when the cytology result was moderate dyskaryosis or worse (>BMD) irrespective of hrHPV status. Participants returned to the next screening round after 5 years if the cytological result was normal and the hrHPV test was negative. Otherwise, participants were followed both cytologically and with hrHPV testing at 6 and 18 months. Participants with BMD at baseline were referred for colposcopy at 6 months if the 6-month result was hrHPV-positive BMD, or >BMD irrespective of hrHPV status, and participants with hrHPV positive normal cytology at intake were referred at 6 months only for >BMD. All participants in the intervention group were referred at 18 months for colposcopy if the combined test suggested the presence of a cervical lesion. The participants in the control group (n = 22,106) followed the current Dutch screening guidelines. Participants were referred for colposcopy if the result was >BMD at intake. Participants returned to the next screening round at 5 years if the result was normal. Smears were repeated at 6 or 18 months in case the result was BMD. All participants in the control group were referred at 18 months for colposcopy if cytology was >BMD.

Conventional cytological smears were taken using a Cervex-Brush (Rovers Medical Devices, Oss, The Netherlands). The brush was placed in a vial containing a collection medium (i.e., 5 ml PBS and 0.5% thiomersal) for hrHPV testing. Cervical smears were classified according to the Dutch CISOE-A classification blinded to hrHPV status of participants. In short, cytological
results were grouped as normal, borderline or mild dyskaryosis (BMD; translating into ASC-US/ASC-H/LSIL) and moderate dyskaryosis or worse ( > BMD; translating into HSIL). Detection of hrHPV was performed by GP5+/6+ PCR enzyme immunoassay, using a cocktail of 14 high-risk types, i.e., HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66 and HPV68.15 HR HPV tests were performed in duplicate, and all hrHPV-positive samples were typed by reverse line blotting.14 Technicians performing hrHPV testing were blinded to the cytology results.

Colposcopically directed biopsies were taken for histological examination when suspected areas on the cervix were present according to standard procedures in The Netherlands, and abnor-
mal results were classified histologically as CIN 1, 2 or 3, or invasive cancer according to international criteria.15,16 We included all lesions diagnosed after the referral smear and within 3 years after baseline. Histology samples were read in a community setting and were not subjected to revision.10

Statistical analysis
All participants received cytological analyses (i.e., Pap tests) and hrHPV testing at baseline. Using the screening results leading to a repeat or referral advice at baseline, we defined groups based on the combinations of cytology (normal, BMD, > BMD) and hrHPV (positive (+)/negative (–)) test result. hrHPV positive samples were further stratified on the presence of HPV18 in the baseline sample, since these 2 types account for 70% of cervical cancer cases. Participants with follow-up were further stratified on cytology or hrHPV test result at 6 months. The outcome of interest was defined as a lesion of at least CIN2 (> CIN2, i.e., CIN2, CIN3 or invasive cancer). All analyses were repeated using lesions > CIN3 as outcome measure. Cumulative 18-month incidences as a measure of risk of lesions > CIN2 were assessed using Kaplan–Meier methods and 95% CI’s were calculated. The 95% CI’s on the original > CIN2 risk scale were obtained by exponentiating the upper and lower bounds of the 95% confidence intervals constructed on the log risk scale. Specific groups were compared using log-rank testing. Reported sensitivities and specificities were adjusted for nonverifi-
cation occurring because women in the control group with normal cytology were sent back to routine screening regardless of the blinded hrHPV test result and because some women were lost to follow-up.15 Because the Medical Ethics Committee did not allow recalling participants with normal cytology and a negative hrHPV test for repeat testing earlier than the regular screening interval of 5 years, we assumed women who were sent back to routine screening not to have an underlying CIN lesion. This assumption does not affect the relative sensitivity.15–19

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

Results
Of 44,102 participants of the POBASCAM trial, we included 763 participants with normal cytology and a positive hrHPV test from the intervention group, 1,080 participants with a BMD result of whom 381 (35.3%) tested hrHPV positive and 350 participants with a > BMD result of whom 313 (89.4%) tested hrHPV positive. Mean age was 38.5 years (range 29–60) for participants with normal cytology and a positive hrHPV test, 40.0 years (range 29–60) for participants with BMD and 37.3 years (range 29–60) for participants with > BMD. The nonresponse rate was 23.1% (165/713) at 6 months and 28.0% (146/522) at 18 months for participants with normal cytology. The nonresponse rates at 6 and 18 months for participants with BMD were 9.9% (37/374) and 28.8% (53/184), respectively.

In the control arm, > CIN2 risk for women with a baseline abnormal smear (i.e., > BMD) was 27% (95% CI: 24–31). In the intervention arm, > CIN2 risk was 26% (95% CI: 23–29) for women with a baseline positive hrHPV test, 28% (95% CI: 24–31) for women with abnormal cytology at baseline and 19% (95% CI: 17–21) for women with normal cytology and a positive hrHPV test. The relative risk of > CIN2 for women with a positive hrHPV test in the intervention arm compared with abnormal cytology in the control arm was 25.6 (2.72 = 0.94 (95% CI: 0.8–1.1). The relative risk of > CIN2 for women with a positive hrHPV test and/or abnormal cytology in the intervention arm compared with abnormal cytology in the control arm was 0.71 (95% CI: 0.60–0.83). Detection rates of > CIN2 in women with BMD did not differ between intervention (14%, 95% CI: 11–18) and control group (13%, 95% CI: 11–17).

Because > CIN2 risks in women with abnormal cytology did not depend on the allocation to either intervention or control group (Fig. 1), risks were pooled in further analyses. Furthermore, only results for > CIN2 are discussed. Results were comparable, albeit with lower absolute risks, using lesions > CIN3 as outcome measure.

In Table 1, separate > CIN2 risks are presented for strata defined by baseline cytology and hrHPV status. The lowest risk of 2.5% (95% CI: 1.5–4.2) was observed in women with BMD and a negative hrHPV test. A low > CIN2 risk of 5.3% (95% CI: 3.3–8.6) was also observed in women with normal cytology who were infected

<table>
<thead>
<tr>
<th>Intake</th>
<th>Total</th>
<th>&gt; CIN2</th>
<th>&gt; CIN3</th>
<th>CxCa</th>
<th>Risk (95%CI)</th>
<th>Risk (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N and HPV +</td>
<td>763</td>
<td>58</td>
<td>29</td>
<td>1</td>
<td>12 (9.7–16)</td>
<td>6.1 (4.2–8.7)</td>
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<tr>
<td>16+ and/or 18+</td>
<td>262</td>
<td>42</td>
<td>23</td>
<td>–</td>
<td>25 (19–33)</td>
<td>13 (8.9–19)</td>
</tr>
<tr>
<td>16+ and/or 18–</td>
<td>501</td>
<td>16</td>
<td>6</td>
<td>1</td>
<td>5.3 (3.3–8.6)</td>
<td>2.2 (1.0–4.8)</td>
</tr>
<tr>
<td>BMD and HPV –</td>
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<td>15</td>
<td>8</td>
<td>2</td>
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<td>1.8 (0.9–3.3)</td>
</tr>
<tr>
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<td>381</td>
<td>108</td>
<td>63</td>
<td>5</td>
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<td>20 (16–25)</td>
</tr>
<tr>
<td>16+ and/or 18–</td>
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<td>59</td>
<td>41</td>
<td>5</td>
<td>43 (35–52)</td>
<td>31 (23–39)</td>
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<tr>
<td>16– and/or 18–</td>
<td>220</td>
<td>49</td>
<td>22</td>
<td>–</td>
<td>26 (20–33)</td>
<td>12 (8.3–18)</td>
</tr>
<tr>
<td>&gt; BMD and HPV –</td>
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<td>16</td>
<td>8</td>
<td>1</td>
<td>49 (34–66)</td>
<td>27 (16–44)</td>
</tr>
<tr>
<td>&gt; BMD and HPV +</td>
<td>313</td>
<td>246</td>
<td>192</td>
<td>9</td>
<td>79 (74–83)</td>
<td>62 (56–67)</td>
</tr>
<tr>
<td>16+ and/or 18–</td>
<td>205</td>
<td>169</td>
<td>137</td>
<td>9</td>
<td>82 (77–87)</td>
<td>67 (61–74)</td>
</tr>
<tr>
<td>16– and/or 18–</td>
<td>108</td>
<td>77</td>
<td>55</td>
<td>–</td>
<td>71 (63–60)</td>
<td>51 (42–61)</td>
</tr>
</tbody>
</table>

+ Indicates a positive hrHPV test and – indicates a negative hrHPV test. The 16+ and/or 18+ indicates an intake hrHPV test positive for either HPV16 or HPV18, and 16– and 18– indicates an intake hrHPV test positive for types other than HPV16 or HPV18. N indicates normal cytology, BMD indicates borderline or mild dyskaryosis, and > BMD indicates moderate dyskaryosis or worse. CIN indicates cervical intra-epithelial neoplasia, and CxCa indicates cervical cancer (i.e., squamous cell carcinoma, adenocarcinoma and adenoscarcinoma in situ).
TABLE II – CUMULATIVE 18-MONTH RISK OF HISTOLOGICALLY DIAGNOSED CIN2 OR WORSE STRATIFIED BY CYTOLOGY AND hrHPV AT BASELINE AND CYTOLOGY AT 6 MONTHS

<table>
<thead>
<tr>
<th>Intake</th>
<th>6 months</th>
<th>&lt;CIN2</th>
<th>≥CIN2</th>
<th>CxCa</th>
<th>Risk (95% CI)</th>
<th>Risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N and HPV+</td>
<td>N</td>
<td>399</td>
<td>14</td>
<td>4</td>
<td>5.5 (3.3–9.2)</td>
<td>1.6 (0.6–4.2)</td>
</tr>
<tr>
<td>BMD</td>
<td>70</td>
<td>16</td>
<td>8</td>
<td>1</td>
<td>27 (17–40)</td>
<td>17 (7.0–25)</td>
</tr>
<tr>
<td>≥BMD</td>
<td>24</td>
<td>19</td>
<td>12</td>
<td>12</td>
<td>79 (62–92)</td>
<td>50 (32–71)</td>
</tr>
<tr>
<td>16+ and/or 18+</td>
<td>N</td>
<td>135</td>
<td>12</td>
<td>3</td>
<td>13 (7.8–22)</td>
<td>3.3 (1.1–10)</td>
</tr>
<tr>
<td>BMD</td>
<td>28</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>52 (34–73)</td>
<td>23 (13–51)</td>
</tr>
<tr>
<td>≥BMD</td>
<td>15</td>
<td>13</td>
<td>11</td>
<td>11</td>
<td>87 (65–98)</td>
<td>73 (50–92)</td>
</tr>
<tr>
<td>16– and 18–</td>
<td>N</td>
<td>264</td>
<td>2</td>
<td>1</td>
<td>1.2 (0.3–4.9)</td>
<td>0.6 (0.1–4.3)</td>
</tr>
<tr>
<td>BMD</td>
<td>42</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>11 (4.2–26)</td>
<td>5.4 (1.4–20)</td>
</tr>
<tr>
<td>≥BMD</td>
<td>9</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>67 (38–92)</td>
<td>11 (1.6–57)</td>
</tr>
<tr>
<td>BMD and HPV+</td>
<td>N</td>
<td>485</td>
<td>–</td>
<td>–</td>
<td>0.0 (0.0–0.8)</td>
<td>0.0 (0.0–0.8)</td>
</tr>
<tr>
<td>BMD</td>
<td>81</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>6.4 (2.7–15)</td>
<td>4.0 (1.3–12)</td>
</tr>
<tr>
<td>≥BMD</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>86 (54–99)</td>
<td>71 (39–96)</td>
</tr>
<tr>
<td>16+ and/or 18+</td>
<td>N</td>
<td>140</td>
<td>13</td>
<td>4</td>
<td>18 (8.5–23)</td>
<td>4.6 (1.7–12)</td>
</tr>
<tr>
<td>BMD</td>
<td>109</td>
<td>41</td>
<td>20</td>
<td>1</td>
<td>38 (29–48)</td>
<td>18 (12–27)</td>
</tr>
<tr>
<td>≥BMD</td>
<td>48</td>
<td>38</td>
<td>26</td>
<td>4</td>
<td>79 (67–89)</td>
<td>54 (41–69)</td>
</tr>
<tr>
<td>16– and 18–</td>
<td>N</td>
<td>52</td>
<td>7</td>
<td>2</td>
<td>21 (11–30)</td>
<td>6.1 (1.6–22)</td>
</tr>
<tr>
<td>BMD</td>
<td>43</td>
<td>20</td>
<td>15</td>
<td>1</td>
<td>46 (33–62)</td>
<td>35 (23–51)</td>
</tr>
<tr>
<td>≥BMD</td>
<td>28</td>
<td>23</td>
<td>16</td>
<td>1</td>
<td>82 (66–94)</td>
<td>57 (40–75)</td>
</tr>
<tr>
<td>16– and 18–</td>
<td>N</td>
<td>88</td>
<td>6</td>
<td>2</td>
<td>11 (4.8–14)</td>
<td>3.8 (1.0–14)</td>
</tr>
<tr>
<td>BMD</td>
<td>66</td>
<td>21</td>
<td>5</td>
<td>5</td>
<td>32 (22–45)</td>
<td>7.6 (3.2–17)</td>
</tr>
<tr>
<td>≥BMD</td>
<td>20</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td>75 (55–91)</td>
<td>50 (31–73)</td>
</tr>
</tbody>
</table>

+ Indicates a positive hrHPV test, and − indicates a negative hrHPV test. 16+ and/or 18+ indicates an intake hrHPV test positive for either HPV16 or HPV18, and 16– and 18– indicates an intake hrHPV test positive for types other than HPV16 or HPV18. N indicates normal cytology, BMD indicates borderline or mild dyskaryosis, and ≥BMD indicates moderate dyskaryosis or worse. CIN indicates cervical intraepithelial neoplasia, and CxCa indicates cervical cancer (i.e., squamous cell carcinoma, adenocarcinoma and adenocarcinoma in situ). Participants without a hrHPV test result at 6 months were excluded from the analyses.

TABLE III – CUMULATIVE 18-MONTH RISK OF HISTOLOGICALLY DIAGNOSED CIN2 OR WORSE STRATIFIED BY CYTOLOGY AND hrHPV AT BASELINE AND hrHPV AT 6 MONTHS

<table>
<thead>
<tr>
<th>Intake</th>
<th>6 months</th>
<th>&lt;CIN2</th>
<th>≥CIN2</th>
<th>CxCa</th>
<th>Risk (95% CI)</th>
<th>Risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N and HPV+</td>
<td>HPV–</td>
<td>165</td>
<td>2</td>
<td>–</td>
<td>2.4 (0.6–9.1)</td>
<td>0.0 (0.0–2.2)</td>
</tr>
<tr>
<td>HPV+</td>
<td>266</td>
<td>46</td>
<td>23</td>
<td>1</td>
<td>20 (15–25)</td>
<td>9.8 (6.6–14)</td>
</tr>
<tr>
<td>HPV–</td>
<td>48</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>7.4 (4.9–26)</td>
<td>0.0 (0.0–7.4)</td>
</tr>
<tr>
<td>HPV+</td>
<td>112</td>
<td>35</td>
<td>20</td>
<td>1</td>
<td>36 (27–46)</td>
<td>20 (13–30)</td>
</tr>
<tr>
<td>HPV–</td>
<td>117</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.0 (0.0–3.0)</td>
<td>0.0 (0.0–3.0)</td>
</tr>
<tr>
<td>HPV+</td>
<td>154</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>7.9 (4.5–14)</td>
<td>2.0 (0.8–7.3)</td>
</tr>
<tr>
<td>HPV–</td>
<td>378</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>1.5 (0.6–3.6)</td>
<td>1.2 (0.4–3.3)</td>
</tr>
<tr>
<td>HPV+</td>
<td>10</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>10 (1.5–53)</td>
<td>0.0 (0.0–31)</td>
</tr>
<tr>
<td>HPV–</td>
<td>55</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>11 (4.7–26)</td>
<td>4.9 (1.2–19)</td>
</tr>
<tr>
<td>HPV+</td>
<td>142</td>
<td>54</td>
<td>32</td>
<td>3</td>
<td>39 (32–48)</td>
<td>24 (17–32)</td>
</tr>
<tr>
<td>HPV–</td>
<td>11</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>24 (6.3–70)</td>
<td>17 (2.5–73)</td>
</tr>
<tr>
<td>HPV+</td>
<td>73</td>
<td>31</td>
<td>23</td>
<td>3</td>
<td>44 (33–57)</td>
<td>33 (23–46)</td>
</tr>
<tr>
<td>HPV–</td>
<td>44</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>8.2 (2.6–24)</td>
<td>2.3 (0.3–1.5)</td>
</tr>
<tr>
<td>HPV+</td>
<td>69</td>
<td>23</td>
<td>9</td>
<td>9</td>
<td>34 (24–46)</td>
<td>14 (7.5–25)</td>
</tr>
</tbody>
</table>

+ Indicates a positive hrHPV test, and − indicates a negative hrHPV test. 16+ and/or 18+ indicates an intake hrHPV test positive for either HPV16 or HPV18, and 16– and 18– indicates an intake hrHPV test positive for types other than HPV16 or HPV18. N indicates normal cytology, BMD indicates borderline or mild dyskaryosis, and ≥BMD indicates moderate dyskaryosis or worse. CIN indicates cervical intraepithelial neoplasia, and CxCa indicates cervical cancer (i.e., squamous cell carcinoma, adenocarcinoma and adenocarcinoma in situ). Participants without a cytology result at 6 months were excluded from the analyses.

with hrHPV but not HPV16 and/or HPV18. In the subsets of hrHPV-positive women with normal cytology and HPV16 and/or HPV18, hrHPV-positive women with BMD, and women with ≥BMD, ≥CIN2 risks were moderate to high. Risks for HPV16-positive women only were comparable with the risk of women with HPV16 and/or 18 (data not shown).

A detailed overview of the role of repeat cytology on ≥CIN2 risks is displayed in Table II, showing ≥CIN2 risks for strata defined by hrHPV and cytology at baseline, and cytology at 6 months. In women with hrHPV-positive normal cytology at baseline, cumulative risk of ≥CIN2 increased from 5.5% (95%CI: 3.3–9.2) to 79% (95% CI: 61–92) when comparing women with a second normal smear with women who had ≥BMD at 6 months. Women who had HPV16 and/or HPV18 at baseline with 2 normal smears had a risk of 13% (95% CI: 7.8–22), whereas women who tested hrHPV-positive for other high-risk types had a much lower risk of 1.2% (95% CI: 0.3–4.9). Risks in HPV16 and/or HPV18-positive women with normal cytology at baseline and BMD at 6 months were 52% (95%CI: 34–73), and 11% (95%CI: 4.2–26) for hrHPV-positive women positive for other types. In women with a hrHPV-negative BMD smear at baseline and cytological regression at 6 months (n = 485), ≥CIN2 risk was 0% (95%CI: 0.0–0.8). In contrast, women with hrHPV-positive BMD and cytological regression still had a substantial risk of ≥CIN2 of 14% (95% CI: 8.5–23). Women with ≥BMD at 6 months irrespective of baseline cytology had high ≥CIN2 risks.

Table III gives a detailed presentation of the role of hrHPV clearance and displays the cumulative risk of a lesion ≥CIN2 stratified for baseline results, and hrHPV test result at 6 months. In women with hrHPV-positive normal cytology at baseline, cumula-
Table IV – Cumulative 18-Month Risk of Histologically Diagnosed CIN2 or Worse Stratified by Cytology and hrHPV at Baseline and Cytology and hrHPV at 6 Months

<table>
<thead>
<tr>
<th>Intake 6 months</th>
<th>≥CIN2</th>
<th>≥CIN3</th>
<th>CxCa</th>
<th>Risk (95% CI)</th>
<th>Risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N and HPV -</td>
<td>155</td>
<td>1</td>
<td>-</td>
<td>1.3 (0.0–2.4)</td>
<td>0.0 (0.0–2.4)</td>
</tr>
<tr>
<td>16+ and/or 18+</td>
<td>274</td>
<td>47</td>
<td>23</td>
<td>19 (15.5–25)</td>
<td>9.5 (6.4–14.0)</td>
</tr>
<tr>
<td>BMD and/or HPV+</td>
<td>112</td>
<td>36</td>
<td>20</td>
<td>37 (23.8–47)</td>
<td>20 (13–30)</td>
</tr>
<tr>
<td>N and HPV -</td>
<td>108</td>
<td>1</td>
<td>-</td>
<td>0.0 (0.0–3.4)</td>
<td>0.0 (0.0–2.4)</td>
</tr>
<tr>
<td>16+ and/or 18+</td>
<td>162</td>
<td>11</td>
<td>3</td>
<td>7.5 (4.2–13.2)</td>
<td>3.0 (2.7–6.9)</td>
</tr>
<tr>
<td>BMD and HPV+</td>
<td>325</td>
<td>-</td>
<td>-</td>
<td>0.0 (0.0–1.1)</td>
<td>0.0 (0.0–1.1)</td>
</tr>
<tr>
<td>N and HPV -</td>
<td>63</td>
<td>6</td>
<td>4</td>
<td>9.4 (5.5–21)</td>
<td>6.7 (2.6–17)</td>
</tr>
<tr>
<td>16+ and/or 18+</td>
<td>42</td>
<td>-</td>
<td>-</td>
<td>0.0 (0.0–8.4)</td>
<td>0.0 (0.0–8.4)</td>
</tr>
<tr>
<td>BMD and/or HPV+</td>
<td>152</td>
<td>57</td>
<td>32</td>
<td>39 (31–47)</td>
<td>22 (16–30)</td>
</tr>
<tr>
<td>N and HPV -</td>
<td>73</td>
<td>31</td>
<td>22</td>
<td>45 (34–57)</td>
<td>31 (22–44)</td>
</tr>
<tr>
<td>16+ and/or 18+</td>
<td>33</td>
<td>-</td>
<td>-</td>
<td>0.0 (0.0–11)</td>
<td>0.0 (0.0–11)</td>
</tr>
<tr>
<td>BMD and/or HPV+</td>
<td>79</td>
<td>26</td>
<td>10</td>
<td>33 (24–45)</td>
<td>14 (7.4–24)</td>
</tr>
</tbody>
</table>

+ Indicates a positive hrHPV test, and – indicates a negative hrHPV test. 16+ and/or 18+ indicates an intake hrHPV test positive for either HPV16 or HPV18, and 16– and/or 18– indicates an intake hrHPV test positive for types other than HPV16 or HPV18. N indicates normal cytology, BMD indicates borderline or mild dyskaryosis, and >BMD indicates moderate dyskaryosis or worse. CIN indicates cervical intra-epithelial neoplasia, and CxCa indicates cervical cancer (i.e., squamous cell carcinoma, adenocarcinoma and adenosquamous carcinoma in situ). Participants without a cytology or hrHPV test result at 6 months were excluded from the analyses.

Table V – Specificity and Sensitivity of Cytology Results and hrHPV Test Result at Baseline at Different Thresholds for Histological Detection of Lesions ≥CIN2 (Cumulative 18-Month Risk)

<table>
<thead>
<tr>
<th>Screening test</th>
<th>Threshold</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytology</td>
<td>&gt; BMD</td>
<td>68.0 (64.2–71.6)</td>
<td>97.7 (97.4–98.1)</td>
<td>75.5 (71.0–79.5)</td>
<td>97.4 (97.2–97.9)</td>
</tr>
<tr>
<td>hrHPV Positive</td>
<td>&gt; BMD</td>
<td>94.1 (91.7–95.9)</td>
<td>99.8 (99.6–100)</td>
<td>94.2 (91.8–95.9)</td>
<td>95.6 (95.5–95.8)</td>
</tr>
<tr>
<td>hrHPV and</td>
<td>&gt; BMD</td>
<td>61.6 (57.7–65.4)</td>
<td>99.3 (99.0–99.5)</td>
<td>96.3 (95.3–97.3)</td>
<td>99.0 (98.8–99.3)</td>
</tr>
<tr>
<td>Cytology</td>
<td>&gt; BMD</td>
<td>39.7 (25.2–31.9)</td>
<td>99.7 (99.5–100)</td>
<td>48.4 (28.4–37.1)</td>
<td>99.6 (99.3–99.9)</td>
</tr>
<tr>
<td></td>
<td>&gt; BMD</td>
<td>22.4 (19.2–25.9)</td>
<td>99.6 (99.3–99.8)</td>
<td>21.3 (17.5–25.6)</td>
<td>99.4 (99.2–99.7)</td>
</tr>
<tr>
<td>hrHPV Positive</td>
<td>&gt; BMD</td>
<td>40.7 (36.9–44.7)</td>
<td>99.8 (99.6–100)</td>
<td>49.6 (45.2–54.0)</td>
<td>99.7 (99.5–100)</td>
</tr>
<tr>
<td>hrHPV and</td>
<td>&gt; BMD</td>
<td>28.2 (24.7–31.9)</td>
<td>99.9 (99.7–100)</td>
<td>35.7 (31.1–40.6)</td>
<td>99.8 (99.6–100)</td>
</tr>
<tr>
<td>Cytology</td>
<td>&gt; BMD</td>
<td>12.8 (10.4–15.8)</td>
<td>99.9 (99.7–100)</td>
<td>14.2 (11.1–18.1)</td>
<td>99.9 (99.6–100)</td>
</tr>
<tr>
<td>hrHPV Positive</td>
<td>Positive or &gt; BMD</td>
<td>100 (reference)</td>
<td>94.5</td>
<td>100 (reference)</td>
<td>94.0</td>
</tr>
<tr>
<td>hrHPV Positive</td>
<td>&gt; BMD</td>
<td>90.8 (88.0–93.0)</td>
<td>96.7 (96.5–96.9)</td>
<td>94.1 (91.9–96.1)</td>
<td>96.3 (96.1–96.5)</td>
</tr>
<tr>
<td>hrHPV Positive</td>
<td>&gt; BMD</td>
<td>78.3 (74.6–81.7)</td>
<td>98.7 (98.4–98.9)</td>
<td>83.8 (79.5–87.3)</td>
<td>98.3 (98.1–98.6)</td>
</tr>
<tr>
<td>hrHPV Positive</td>
<td>Positive or &gt; BMD</td>
<td>97.1 (95.2–98.3)</td>
<td>96.0 (95.9–96.1)</td>
<td>96.7 (94.8–98.0)</td>
<td>95.6 (95.5–95.7)</td>
</tr>
<tr>
<td>hrHPV Positive</td>
<td>&gt; BMD</td>
<td>77.2 (73.6–80.5)</td>
<td>95.4 (95.3–95.5)</td>
<td>81.4 (77.1–85.1)</td>
<td>95.1 (94.5–95.2)</td>
</tr>
<tr>
<td>hrHPV Positive</td>
<td>&gt; BMD</td>
<td>62.8 (58.7–66.7)</td>
<td>97.2 (97.0–97.3)</td>
<td>65.5 (60.5–70.2)</td>
<td>96.9 (96.7–97.1)</td>
</tr>
</tbody>
</table>

N indicates normal cytology, BMD indicates borderline or mild dyskaryosis, and >BMD indicates moderate dyskaryosis or worse. NA indicates not applicable. Sensitivity is defined relative to the sensitivity of women with abnormal cytology and a positive hrHPV test (reference group), respectively.

tive risk of ≥CIN2 was 2.4% (95% CI: 0.6–9.1) in women testing negative for hrHPV at 6 months and 20% (95% CI: 15–25) in women who tested hrHPV-positive twice. In the group of hrHPV-positive women with normal cytology at baseline and a negative hrHPV test at 6 months, all ≥CIN2 cases diagnosed tested positive for HPV16 and/or HPV18 at baseline. For the other strata with a negative hrHPV test at 6 months, ≥CIN2 risks were low but not negligible.

Finally, we evaluated the cumulative risk of a lesion ≥CIN2 based on hrHPV status and cytology at baseline and at 6 months (Table I). Overall, the ≥CIN2 risk of women with a hrHPV-negative normal smear at 6 months (n = 522) was 0.2% (95% CI: 0.0–0.8). In women with hrHPV-positive normal cytology at baseline, cumulative risk of ≥CIN2 was 1.3% (95% CI: 0.0–2.4) in women double negative at 6 months and 19% (95% CI: 15–25) in women who tested positive for cytology and/or hrHPV at 6 months. In the group of hrHPV-positive women without HPV16 and/or HPV18 at baseline with a hrHPV-negative normal test at 6 months, no cases of ≥CIN2 were diagnosed. In women with hrHPV-negative BMD at baseline, risk was 0.0% (95% CI: 0.0–1.1) in women who had a hrHPV-negative normal smear at 6 months and 9.8% (95% CI: 4.5–21) in women positive for either test at 6 months. Women with BMD cytology and a positive hrHPV test at baseline that tested hrHPV-negative with normal cytology at follow-up had a risk of
0.0% (95%CI: 0.0–8.4) and who tested positive for cytology and/or hrHPV had a risk of 39% (95%CI: 31–47).

To evaluate the test characteristics of cytology and hrHPV screening, we calculated sensitivity and specificity for different thresholds of test positivity (Table V). Since lesions could only have been detected in case of either ≥BMD or hrHPV positivity, the sensitivity of combined testing was assumed to be 100%. Using a threshold of ≥BMD, sensitivity for the detection of lesions ≥CIN2 was 68.0% (95%CI: 64.2–71.6) and specificity was 97.7% (95%CI: 97.4–98.1). Using hrHPV positivity as threshold, the sensitivity was 94.1% (95%CI: 91.7–95.9) and specificity was 96.1% (95%CI: 96.0–96.1). With hrHPV positivity for HPV16 and/or HPV18 as threshold, sensitivity was 62.5% (95%CI: 58.1–66.7) and specificity was 98.8% (98.6–99.0). The relative sensitivity of hrHPV testing compared with cytological testing was 0.94/0.680 = 1.38 (95%CI: 1.25–1.56) and the relative specificity was 0.96/0.977 = 0.98 (95%CI: 0.98–0.99).

Using both ≥BMD and hrHPV positivity as threshold, estimates for sensitivity decreased slightly to 61.6% (95%CI: 57.7–65.4) for hrHPV-positive ≥BMD compared with a ≥BMD cytology threshold, and specificity increased substantially to 99.3% (95%CI: 99.0–99.5). Using a positive test result of either test as threshold in which hrHPV positivity was assumed to be 100%, the specificity was 94.5%.

### Discussion

In this implementation study of hrHPV testing in population-based screening, we showed that primary hrHPV testing is more sensitive than cytology to detect ≥CIN2 lesions at the cost of slightly lower specificity. Moreover, we showed that women with HPV16 and/or HPV18 have a much higher ≥CIN2 risk than women positive for another hrHPV type. Retesting at 6 months showed that both cytological regression and hrHPV clearance are associated with decreased risks of ≥CIN2. Interestingly, women who had a double negative test at 6 months had a ≥CIN2 risk of 0.2% (95%CI: 0–1.1) and women with a hrHPV-negative BMD smear at baseline and normal cytology at 6 months had a ≥CIN2 risk of 0.0 (95%CI: 0–0.8). These data show that the risk of ≥CIN2 is better identified by 6 months with cytology and/or hrHPV testing than by single visit testing.

Finally, our data support colposcopy referral for women with either hrHPV-positive BMD, or ≥BMD regardless of hrHPV status because of the magnitude of the risk of ≥CIN2.

In this study, retesting moments were 6 and 18 months as mandatory in the Dutch cervical screening programme. If hrHPV testing is implemented in primary screening, other retesting moments might be more cost-effective, with for example shorter intervals for HPV16 and HPV18 and longer intervals for other hrHPV types.

With lesions ≥CIN2 as the outcome of interest, screening sensitivity of classical cytology at a threshold of BMD was 68.0% and specificity was 97.7%. The sensitivity increased to 94.1% when using hrHPV testing, and specificity became slightly lower (96.1%).

These data are in line with previous estimates obtained in screening studies using classical cytology and hrHPV testing by either GP5+/6+ PCR or Hybrid Capture 2.21,28 and support the opinion that hrHPV testing should be used either alone or in conjunction with cytology in cervical screening. We also showed that the risk of ≥CIN2 in women with HPV18 infection and normal cytology is higher than in women with hrHPV-negative BMD (12% vs. 2.5%). Besides, women with normal cytology harboring HPV16 and/or HPV18 had a substantially higher risk of ≥CIN2 than women infected with another high-risk type (25% vs. 5.5%), although the ≥CIN2 risk of the latter group was still higher than in the group of women with a hrHPV-negative BMD smear. Similar data have been described by Khan et al. and Berkhof et al.25,26 These results underline that hrHPV-positive women with normal cytology, and especially women with HPV16 or HPV18, should have shorter intervals for retesting than women with BMD and a hrHPV-negative test result.

We assume that among women with an abnormal smear or a positive hrHPV test at baseline the vast majority of ≥CIN2 lesions diagnosed during the study were prevalent, and the histological diagnosis was postponed due to the screening algorithm as women with BMD or normal cytology were not referred for colposcopy immediately. Thus, a difference between prevalent cases diagnosed at intake and incident cases only diagnosed during follow-up was not made. Several studies, using either histological or cytological data, have indicated that disease detected during short-term follow-up corresponds to “missed” prevalent disease.24,27

Until now most studies evaluated the risk of ≥CIN2 for strata defined by hrHPV test results and cytology at baseline.2,8,18,24–27 Our study shows that for women with BMD or hrHPV-positive normal cytology a second test at 6 months, whether it be cytology or hrHPV testing, is more accurate in detecting ≥CIN2 than a single test at baseline. Tailoring the follow-up to allow for clearance of hrHPV and cytological regression of lesions will lead to a decrease in referrals for colposcopy. This is especially useful for hrHPV-positive women with normal cytology, since their baseline risk of a high-grade lesion is moderate. By retesting at 6 months, women can be distinguished with either a high or low ≥CIN2 risk. For instance, in our study HPV16 and/or HPV18-positive women with normal cytology at baseline followed by abnormal cytology at 6 months had a 4-fold higher risk of ≥CIN2 than women with normal cytology at 6 months. In the group of hrHPV-positive women without HPV16 and/or HPV18, the risk of ≥CIN2 was 9-fold higher when the repeat test was ≥BMD compared with normal. Results were even more pronounced when distinguishing women with a hrHPV-negative normal smear at 6 months as they had virtually no ≥CIN2 risk.

Although the negative predictive value for ≥CIN2 after a negative hrHPV test at baseline is higher than after negative cytology, the risk of a high-grade cervical lesion is not completely absent. Some participants in our study had high-grade cervical lesions but a negative hrHPV test (n = 31), a phenomenon also found by others.22,23 These failures may be attributable to failure of cervical cell sampling, false-negative hrHPV test results or possibly incident disease. Additional analyses of samples from participants with ≥CIN2 that tested negative for hrHPV using the crude sample indicate that approximately half of the samples were negative due to inadequate material for PCR. The remaining samples were positive by E7 PCR, suggesting that integration of hrHPV DNA caused a negative GPS+6+/PCR result (data not shown).

In conclusion, hrHPV is a major risk factor of high-grade cervical lesions and cervical cancer. We have now shown that repeat testing for women with BMD or hrHPV-positive normal cytology using either cytology or hrHPV testing detects the risk of ≥CIN2 better than single visit testing, and that HPV16 and/or HPV18 identifies women with the highest risk of ≥CIN2. Moreover, in women with hrHPV-negative normal cytology, the second test the risk of ≥CIN2 is virtually absent. At present, we are conducting cost-effectiveness analyses to determine the optimal algorithm for the use of cytology and hrHPV testing to detect cervical cancer and its precursor lesions in cervical screening programmes.

### Acknowledgements

We gratefully acknowledge the work of the 242 GPs and their assistants, the District Health Authority Amstelveen, Medial, and District Health Authority Kennemerland-Haarlemmermeer e.o. We especially thank the research analysts of the Unit Molecular Pathology (VU University Medical Center, Amsterdam) and the Cytootechnologists (Spaarte Ziekenhuis, Hoofddorp; Kennemer Gasthuis, Haarlem; Leiden Cytology and Pathology Laboratory, Leiden; Unit Cytopathology, VU University Medical Center, Amsterdam).


Chapter 8

High-risk HPV type-specific clearance rates in cervical screening
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British Journal of Cancer 2007; 96(9): 1419-1424
High-risk HPV type-specific clearance rates in cervical screening

We assessed clearance rates of 14 high-risk human papillomavirus (hrHPV) types in hrHPV-positive women with normal cytology and borderline/mild dyskaryosis (BMD) in a population-based cervical screening cohort of 44,102 women. The 6-month hrHPV type-specific clearance rates, that is, clearance of the same type as detected at baseline, in women with normal and BMD smears were 43% (95% confidence interval (CI) 39–47) and 29% (95% CI 24–34), respectively. Corresponding 18-month clearance rates were markedly higher; namely 65% (95% CI 60–69) and 41% (95% CI 36–47), respectively. The lowest clearance rates in women with normal cytology were for HPV16, HPV18, HPV31, and HPV33. Significantly reduced 18-month clearance rates at a significance level of 1% were observed for HPV16 (49%, 95% CI 41–59) and HPV31 (50%, 95% CI 39–63) in women with normal cytology, and for HPV16 (19%, 95% CI 12–29) in women with BMD. Among women who did not clear hrHPV, women with HPV16 persistence displayed an increased detection rate of ≥CIN3 (normal P<0.0001; BMD, P=0.005). The type-specific differences in clearance rates indicate the potential value of hrHPV genotyping in screening programs. Our data support close surveillance (i.e. referral directly, or within 6 months) of women with HPV16 and are inconclusive for surveillance of women with HPV18, HPV31, and HPV33. For the other hrHPV-positive women, it seems advisable to adopt a conservative management with a long waiting period, as hrHPV clearance is markedly higher after 18 months than after 6 months and the risk for ≥CIN3 is low.

Keywords: human papillomavirus; clearance; type; cervical screening; cervical intraepithelial neoplasia

Persistent infection with high-risk human papillomavirus (hrHPV) is the primary cause for the development of cervical carcinoma (Walboomers et al, 1999; Bosch et al, 2002). Several studies have shown that hrHPV testing can improve identification of women who have or will develop high-grade cervical intraepithelial neoplasia or cervical cancer (≥CIN2) (Clavel et al, 2001; Kulasingam et al, 2002; Cuzick et al, 2003; Peto et al, 2004; Kotaniemi-Talonen et al, 2005; Cuzick et al, 2006).

In the search of an optimal screening algorithm using hrHPV testing, it is essential to determine the time point at which the majority of the screening participants has cleared the virus and can be referred back to regular screening. Also, future screening may involve genotyping as different hrHPV types show markedly different risks of high-grade CIN. Particularly HPV16-positive women are more likely to develop ≥CIN2 than hrHPV-positive women infected with a non-HPV16 type (Bulkmans et al, 2005; Castle et al, 2005; Khan et al, 2005; Berkhold et al, 2006). So far, little is known about type-specific clearance rates of hrHPV infections. Some studies reported relatively low clearance of HPV16 infections compared with other high-risk HPV infections, but samples sizes were small and results were not statistically significant (Ho et al, 1998; Molano et al, 2003; Richardson et al, 2003).

To obtain information about the course of 14 different hrHPV types, we investigated repeated hrHPV typing results collected from a large population-based screening cohort. We assessed differences among the 14 hrHPV types in clearance and in the occurrence of ≥CIN3.

MATERIALS AND METHODS

Study population
From January 1999 to September 2002, 44,102 women between 30 and 60 years of age invited for the regular Dutch cervical screening programme participated in the Population-Based Screening Amsterdam (POBASCAM) trial (Bulkmans et al, 2004). In this prospective randomised controlled trial, the efficacy of hrHPV testing in conjunction with cytology (intervention group) is compared with that of classical cytology alone (control group, hrHPV results blinded) in the setting of population-based cervical screening. The design of the POBASCAM trial and the baseline results have been described previously (Bulkmans et al, 2004). All women gave informed consent and the study was approved by both the Medical Ethics Committee of the VU University Medical Center (no. 96/103) and the Ministry of Public Health (VWS, no. 328650). The study has been registered at the International Trial Register (ISRCTN20781131).
We included all hrHPV-positive participants who were advised to return for repeat testing at 6 and 18 months according to the study design, that is, participants with normal cytology from the intervention group (n = 763) and participants with borderline or mild dyskaryosis (BMD) from the intervention (n = 185) and control (n = 196) group. Borderline/mild dyskaryosis is equivalent to ASC-US, ASC-H, LSIL, AGC and AGC favour neoplastic, according to the Bethesda 2001 classification (Bulk et al., 2004). Women who were positive for hrHPV by generic hrHPV test, but negative after typing, were excluded from the analysis (n = 57), leaving 713 hrHPV-positive participants with normal cytology carrying 865 hrHPV infections, and 374 hrHPV-positive participants with BMD carrying 491 hrHPV infections.

For all included participants, hrHPV testing and typing were performed both at baseline and at 6 and 18 months of follow-up. Participants with a baseline normal smear were referred for colposcopy at 6 months in case the repeat smear displayed moderate dyskaryosis or worse (>BMD; equivalent to HSIL according to Bethesda 2001; Bulk et al., 2004). Participants with baseline BMD were referred for colposcopy at 6 months in case the repeat smear result was >BMD in the control group and either >BMD or hrHPV-positive BMD in the intervention group. A flowchart of the screening management of women who were advised to return for repeat testing is presented in Figure 1.

Cytology and hrHPV testing

Conventional cytological smears were prepared with a Cervex brush® and classified according to the Dutch CISOE-A classification, which can be translated to the Bethesda 2001 classification (Bulk et al., 2004). After taking the smear, the brush was placed in a vial containing collection medium (i.e. 5 ml PBS and 0.5% thiomersal) for hrHPV-DNA testing. Detection of hrHPV-DNA was performed by a generic hrHPV test, that is, GP5+/6+ PCR-enzyme immunoassay (GP5+/6+ PCR EIA), using a cocktail of 14 high-risk types, that is, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 (Jacobs et al., 1997). GP5+/6+ PCR EIA-positive cases were subsequently typed by reverse line blotting (RLB) (van den Brule et al., 2002). Interpretation of cytology and hrHPV testing by technicians was performed blinded to the other test result.

Statistical analysis

We calculated the clearance rates of 14 hrHPV types with type-specific clearance defined as a negative RLB test result for that HPV type in the follow-up smear. Women treated for ≥CIN2 were considered not to have cleared the virus during the study period of 18 months. Participants were censored if lost to follow-up, or if a biopsy was taken with CIN0/1 as histological outcome. Time was set equal to the target repeat date (i.e. 6 or 18 months). The 18-month clearance rates were estimated by Kaplan–Meier method. The clearance rates were accompanied by 95% confidence intervals (95% CI) (Klein and Moeschberger, 1997). Data were stratified in three age categories corresponding to the age at the first round, second round, and at rounds 3–7 in nationwide screening (i.e. 29–33, 34–38, and 39–60 years). Differences in 6-month clearance rates were assessed by Cochran’s Mantel–Haenszel test and differences in 18-month clearance rates were assessed by stratified log-rank testing (Heimann and Neuhaus, 1998). The level of statistical significance was set at 0.01. The main analyses did not distinguish whether the hrHPV infection was observed in a woman with single or multiple hrHPV types. Analyses were repeated for women with single hrHPV infections only. To examine an effect of coexisting hrHPV infections on clearance, type-specific clearance rates in single and multiple infections were compared by stratified log-rank testing. For clinical practice it might be more feasible to define clearance as a negative hrHPV follow-up test result for any hrHPV type. Therefore, analyses were repeated with clearance defined as a negative generic hrHPV test result.

To assess the hrHPV type-specific risk for ≥CIN3 in women who did not clear the hrHPV infection (ie. viral persistence), participants were selected that revealed RLB positivity during follow-up for at least one hrHPV type detected at baseline. The

**Figure 1** Management of women in the POBASCAm study who were advised to return for repeat testing at 6 and 18 months.

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Chapter 8 hrHPV type-specific clearance rates

hrHPV type-specific clearance rates in screening

NWJ Bulkmans et al

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association between persistent hrHPV type and ≥CIN3 was assessed by Cochran's Mantel–Haenszel test stratified for age. To examine the effect of coexisting hrHPV infections, ≥CIN3 rates in women with single and multiple hrHPV infections were compared by Cochran's Mantel–Haenszel testing, stratified for age, cytology, and HPV-type. All analyses were performed using SPSS12.0.

RESULTS
Study subjects
The mean ages of hrHPV-positive women with normal cytology and BMD at baseline were 38.3 years (range 29–60 years) and 36.2 years (range 29–59 years), respectively. Of women with normal cytology, 23.1% (165/713) did not respond to the follow-up invitation at 6 months and 28.0% (146/522) did not respond to the second follow-up invitation at 18 months. For women with BMD, the nonresponse rates to follow-up invitations at 6 and 18 months were 9.9% (37/374) and 28.8% (53/184), respectively. Loss to follow-up was not hrHPV type specific (P > 0.05 for each type). Multiple hrHPV infections were less prevalent in women with normal cytology than in women with BMD (18.0% vs 24.6%, P = 0.011).

Type-specific clearance rates for hrHPV infections in normal cytology
The 6- and 18-month clearance rates of the different hrHPV types in women with normal cytology at baseline are presented in Table 1. The overall type-specific hrHPV clearance rates at 6 and 18 months were 43% (95% CI 39–47) and 65% (95% CI 60–69). HPV16 infections displayed a significantly lower 18-month clearance rate (49%, 95% CI 41–59) than other hrHPV infections (69%, 95% CI 65–74; P = 0.002). The second, third, and fourth lowest 18-month clearance rates were observed for HPV31, HPV33, and HPV18 infections, respectively, but the clearance rate was significantly reduced only for HPV31 (P = 0.008). When comparing single and multiple infections at baseline, none of the hrHPV types showed marked differences in clearance rates (data not shown). Notably, 18-month clearance for HPV16 was 48% (95% CI 38–58) in women with a single infection and 56% (95% CI 39–75) in women with a multiple infection (P = 0.310).

When defining clearance as a negative generic hrHPV test result instead of a negative RLB result for a specific hrHPV type, overall hrHPV clearance rates at 6 and 18 months were slightly lower, that is, 36% (95% CI 31–41) and 36% (95% CI 52–60), respectively. The lowest clearance rates were again found for HPV16, HPV18, HPV31, and HPV33 infections.

Table 1  hrHPV-type specific 6- and 18-month clearance rates for hrHPV infections in normal smears

<table>
<thead>
<tr>
<th>hrHPV type at baseline</th>
<th>N</th>
<th>% (95% CI)</th>
<th>P-value</th>
<th>18 months</th>
<th>% (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>210</td>
<td>16</td>
<td>34 (26–42)</td>
<td>0.018</td>
<td>49 (41–59)</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>18</td>
<td>41 (27–57)</td>
<td>0.271</td>
<td>55 (46–67)</td>
<td>0.032</td>
<td></td>
</tr>
<tr>
<td>111</td>
<td>31</td>
<td>32 (22–45)</td>
<td>0.024</td>
<td>50 (39–63)</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>33</td>
<td>19 (14–28)</td>
<td>0.039</td>
<td>50 (37–64)</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>39</td>
<td>30 (27–47)</td>
<td>0.190</td>
<td>72 (52–92)</td>
<td>0.243</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>42</td>
<td>45 (39–52)</td>
<td>0.046</td>
<td>89 (70–108)</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>53</td>
<td>30 (25–55)</td>
<td>0.148</td>
<td>69 (53–84)</td>
<td>0.229</td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>50</td>
<td>29 (24–37)</td>
<td>0.035</td>
<td>29 (20–41)</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>52</td>
<td>44 (28–63)</td>
<td>0.687</td>
<td>67 (51–82)</td>
<td>0.991</td>
<td></td>
</tr>
<tr>
<td>71</td>
<td>56</td>
<td>42 (27–58)</td>
<td>0.896</td>
<td>79 (59–100)</td>
<td>0.275</td>
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</tr>
<tr>
<td>48</td>
<td>58</td>
<td>53 (37–70)</td>
<td>0.234</td>
<td>78 (64–91)</td>
<td>0.175</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>59</td>
<td>60 (38–85)</td>
<td>0.176</td>
<td>83 (59–107)</td>
<td>0.143</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>66</td>
<td>50 (31–65)</td>
<td>0.347</td>
<td>81 (58–104)</td>
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<td></td>
</tr>
<tr>
<td>36</td>
<td>68</td>
<td>67 (30–90)</td>
<td>0.246</td>
<td>86 (55–99)</td>
<td>0.258</td>
<td></td>
</tr>
</tbody>
</table>

Significant P-values are indicated in bold. CI = confidence interval, hrHPV = high-risk human papillomavirus.

Type-specific clearance rates for hrHPV infections in BMD
For hrHPV infections in women with a BMD smear at baseline, 6 and 18-month type-specific clearance rates were 29% (95% CI 24–34) and 41% (95% CI 36–47) (Table 2). As was the case for women with normal cytology, HPV16 infections in women with BMD showed a significantly reduced 18-month clearance rate (19%, 95% CI 12–29) compared with other hrHPV infections (49%, 95% CI 43–56; P < 0.0001). For HPV31 and HPV33 infections in BMD, clearance rates were also low, but rates were not significantly lower than for other hrHPV infections. Similar clearance rates were observed when the analysis was repeated for women with single infections only. None of the hrHPV types showed marked differences in clearance rates when comparing single infections at baseline to multiple infections at baseline (data not shown).

When clearance was defined as a negative generic hrHPV test instead of a negative RLB test for a specific hrHPV type, hrHPV clearance rates at 6 and 18 months were 25% (95% CI 18–31) and 32% (95% CI 26–38), respectively. The three types with the lowest 18-month clearance rates were again HPV16, HPV31, and HPV33.

High-grade lesions in women with persistent hrHPV infections
The 18-month hrHPV type-specific detection rate of ≥CIN3 in women who showed persistence for at least one hrHPV type was

![chart](chart.png)
DISCUSSION

In our population-based screening cohort, we studied the type-specific clearance in women with an hrHPV infection. Overall 18-month clearance rates in women with normal cytology were about 1.5 times higher than those in women with BMD (65 vs 41%). Besides, about one-third of the women who cleared the virus within 18 months showed clearance between 6 and 18 months. The lowest clearance rates were observed for HPV16, HPV18, HPV31, and HPV33. Only HPV16 and HPV31 were statistically distinct from the other hrHPV types in women with normal cytology, and only HPV16 and HPV31 were statistically distinct in women with BMD. The relatively low clearance rate of HPV16 could also be reflected in earlier studies (Ho et al., 1998; Molano et al., 2003; Richardson et al., 2003). However, in these studies, sample sizes were generally smaller, and either results did not reach statistical significance or many HPV types were grouped together. Furthermore, we found that among women who did not clear the HPV infection, HPV16-positive women displayed a single hrHPV infection than in women with multiple hrHPV infections at baseline. This finding is in accordance with data of other studies, which showed that multiple hrHPV infections decrease in prevalence when comparing hrHPV-positive normal cervices to increasing grades of cervical premalignant disease (Lungu et al., 1992; Sasagawa et al., 2001; An et al., 2003).

Previously described data showed that besides HPV16, HPV18, HPV31, and HPV33 also conferred an increased risk of ≥CIN3 (Castle et al., 2005; Khan et al., 2005; Berkhof et al., 2006). This likely reflects the combined effects of differences in persistence and oncogenic potential of these types compared with other types and finds support by our data. Whereas HPV16 displayed markedly decreased clearance rates and increased ≥CIN3 rates in case of persistence in both women with normal cytology and BMD, HPV18, HPV31, and HPV33 showed some, but less pronounced, effect on one or both of these parameters. The effect was either limited to women with normal cytology or to women with BMD, or, in case of ≥CIN3 rate, only evident after excluding the women having HPV16 infections. The relatively small size of the subgroup of women with type-specific persistence of non-HPV16 types is a likely reason that effects of these types on ≥CIN3 rate were only marginal.

Distinguishing hrHPV types with a decreased clearance rate may have implications for future screening algorithms. When considering implementation of hrHPV genotyping in cervical screening, it is important to evaluate the time point at which genotyping is performed. Genotyping may well be cost-effective when it is limited to baseline samples, and generic hrHPV testing is applied during follow-up. We also calculated clearance rates when clearance was defined as a negative generic hrHPV test instead of a negative RLB result, and it appeared that clearance rates were limited to baseline samples, and generic hrHPV testing is

**Table 2**  hrHPV type-specific 6- and 18-month clearance rates for hrHPV infections in BMD smears

<table>
<thead>
<tr>
<th>N</th>
<th>hrHPV type at baseline</th>
<th>% (95% CI)</th>
<th>P-value</th>
<th>% (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>123</td>
<td>16</td>
<td>9 (5–17)</td>
<td>&lt;0.0001</td>
<td>19 (14–27)</td>
<td>&lt;0.0001</td>
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<td>42</td>
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<td>35</td>
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<td>33 (20–56)</td>
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<tr>
<td>23</td>
<td>39 (8–60)</td>
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<td>19 (5–50)</td>
<td>0.018</td>
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</tr>
<tr>
<td>32</td>
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<td>75</td>
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<tr>
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<tr>
<td>49</td>
<td>64 (45–89)</td>
<td>0.023</td>
<td>31 (19–57)</td>
<td>0.007</td>
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</tr>
<tr>
<td>11</td>
<td>66 (45–89)</td>
<td>0.023</td>
<td>31 (19–57)</td>
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<td>31 (19–57)</td>
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<tr>
<td>54</td>
<td>66 (45–89)</td>
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<td>31 (19–57)</td>
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<tr>
<td>491</td>
<td>Any</td>
<td>29 (24–34)</td>
<td>0.681</td>
<td>29 (24–34)</td>
<td>0.681</td>
</tr>
</tbody>
</table>

Table 2 hrHPV type-specific 6- and 18-month clearance rates for hrHPV infections in BMD smears

Significant P-values are indicated in bold. CI = confidence interval. hrHPV = high-risk human papillomavirus; BMD = borderline-malignant dysplasia.
follow-up time point should not only be targeted on the hrHPV clearance time but also on the risk of \( \geq \text{CIN3} \) for the different HPV types (Berkhof et al, 2006). Notably, women with either BMD or an HPV16-positive normal smear have a clearly increased risk of \( \geq \text{CIN3} \), and should be recalled early or perhaps even referred for colposcopy at baseline. Owing to the small size of the subgroups, our data are inconclusive concerning the surveillance of women with normal cytology and an HPV18, HPV31, or HPV33 infection. For hrHPV-positive women with normal cytology and without HPV16, HPV18, HPV31, and HPV33, a conservative management with repeat testing at a later time point seems feasible. Currently, our data are incorporated in cost-effectiveness studies to assess the optimal algorithm for the follow-up of hrHPV-positive women with normal cytology or BMD.

### ACKNOWLEDGEMENTS

The study was funded by ZON, Zorg Onderzoek Nederland (Netherlands Organisation for Health Research and Development; Grant 30-05220). We gratefully acknowledge the work of the 242 GPs and their assistants, the District Health Authority Amstelveen, Medial, and DHV Kennermern-Laaremmermeer e.o. We thank the research analysts of the Unit Molecular Pathology, VU University Medical Center, Amsterdam for hrHPV testing and HPV typing, and the cytotechnologists (Spaarne Ziekenhuis, Hoofddorp; Kennemer Gasthuis, Haarlem; Leiden Cytology and Pathology Laboratory, Leiden; and Unit Cytopathology, VU University Medical Center, Amsterdam) for cytological testing.

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Appendix

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Chapter 9

Cross-sectional comparison of an automated hybrid capture 2 assay and the consensus GP5+/6+-PCR method in a population-based cervical screening program

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*Journal of Clinical Microbiology* 2006; 44(10): 3680-3685
Cross-Sectional Comparison of an Automated Hybrid Capture 2 Assay and the Consensus GP5+/6+ PCR Method in a Population-Based Cervical Screening Program

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Received 4 October 2005/Returned for modification 6 December 2005/Accepted 6 August 2006

In this cross-sectional study, clinical performances of the hybrid capture 2 assay using an automated instrument (i.e., rapid capture system) (hc2-RCS) and the high-risk human papillomavirus (hrHPV) PCR-enzyme immunoassay (EIA) test were compared using cervical scrape specimens from 8,132 women that participated in a population-based screening trial. The hc2-RCS test scored significantly more samples positive (6.8%) than the GP5+/6+ PCR-EIA (4.8%) (P < 0.0005). This could be attributed largely to a higher positivity rate by the hc2-RCS test for women with cytologically normal, borderline, or mild dyskaryosis. A receiver operating characteristics analysis of the semiquantitative hc2-RCS results in relation to different cytology categories revealed that these differences are owing to differences in assay thresholds. For women classified as having moderate dyskaryosis or worse who also had underlying histologically confirmed cervical intraepithelial neoplasia grade 3 or cervical cancer (≥CIN3), the hc2-RCS scored 97% (31/32) of samples positive, versus 91% (29/32) by GP5+/6+ PCR-EIA. However, this difference was not significant (P = 0.25). After increasing the hc2-RCS cutoff from 1.0 to 2.0 relative light units/cutoff value of the HPV16 calibrator (RLU/CO), no additional CIN3 lesions were missed by hc2-RCS, but the number of test-positive women with normal, borderline, or mild dyskaryosis was significantly decreased (P < 0.0005). However, at this RLU/CO, the difference in test positivity between hc2-RCS and the GP5+/6+ PCR-EIA was still significant (P = 0.02). The use of an RLU/CO value of 3.0 revealed no significant difference between hc2-RCS and GP5+/6+ PCR-EIA results, and equal numbers of smears classified as ≥CIN3 (i.e., 29/32) were detected by both methods. In summary, both assays perform very well for the detection of ≥CIN3 in a population-based cervical screening setting. However, adjustment of the hc2-RCS threshold to an RLU/CO value of 2.0 or 3.0 seems to produce an improved balance between the clinical sensitivity and specificity for ≥CIN3 in population-based cervical screening.

Nowadays, the role of a persistent infection with high-risk human papillomavirus (hrHPV) in the development of cervical cancer is undisputed (2, 18, 29). As a result, multiple studies have investigated the value of adding an hrHPV DNA test to the classical Pap smear to improve the efficacy of cervical cancer screening programs, the triage of women with ambiguous or borderline cervical smears, and the monitoring of women after treatment for high-grade cervical intraepithelial neoplasia (CIN) (5, 10, 30, 31).

However, only a limited number of assays that detect DNA of hrHPV types as a pool have proven to be of clinical value in longitudinal studies involving large cohorts of women. One of these involves the commercially available, FDA-approved hybrid capture 2 (hc2) test. This assay is based on the hybridization of target DNA with a cocktail of full-length RNA probes of 13 hrHPV types, which has an analytical sensitivity of about 450,000 human papillomavirus (HPV) copies per cervical scrape suspension (26). Digene recently introduced a rapid capture system (RCS) allowing high-throughput hc2 testing for population screening in an automated format. The hc2-RCS is a programmable 96-well microplate processor that integrates liquid handling, plate handling, incubation, shaking, and washing via software specifically designed to run the hc2 assay.

Another clinically validated hrHPV detection assay involves the hrHPV GP5+/6+ PCR-EIA, which tests for 14 hrHPV types in one assay in which PCR products are ultimately hybridized to a mixture of specific oligonucleotides. The application of the GP5+/6+ PCR-EIA assay on crude extracts has an analytical sensitivity which is estimated to be in the range of about 1,000 HPV copies per cervical scrape, with variations of about 10-fold, depending on the HPV type (24, 27). This assay has the advantage that direct genotyping is possible on the hrHPV-specific PCR products by reverse line blot analysis (27). Both hc2 and GP5+/6+ PCR show good to excellent interlaboratory reproducibility (8, 14). Despite their good clinical performances in terms of sensitivity and specificity in detecting cervical intraepithelial neoplasia grade 3 lesions or cervical cancer (≥CIN3) (5, 17, 20–22), the two methods have not been directly compared in large population-based studies. This is of particular importance, since there is now compelling evidence that there exists a differential risk posed by the different hrHPV types for cervical cancer (1, 4, 15). These findings ask for HPV typing as a follow-up test to distinguish those hrHPV-positive women that would benefit from more aggressive management on the basis of the HPV type present. Therefore, when hrHPV testing would be implemented in screening pro-

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grams, the advantages of the hc2-RCS (i.e., high-throughput and automated handling) and GP5+/6+ PCR (i.e., easy genotyping) assays could be combined in a combination test in which hc2-RCS is applied first and then GP5+/6+ PCR genotyping as a reflex test on hc2-RCS-positive samples.

This cross-sectional study involved a two-way comparison of the hc2-RCS and GP5+/6+ PCR hrHPV test on cervical scrapings in relation to the cytological results and histological outcome, the latter for women referred for colposcopy because of a cytology reading of moderate dyskaryosis or worse. To that end, cervical scrape specimens from 8,132 women that participated in an extension of a population-based screening (i.e., POBASCAM [5]) trial were analyzed by both methods. To ultimately explain discrepant test results between hc2-RCS and GP5+/6+ PCR, a further comprehensive analysis was performed involving possible PCR inhibition and viral parameters, such as viral type distribution and viral load.

Although the hc2-RCS test showed an overall higher positivity rate than the GP5+/6+ PCR-EIA, particularly cervical scrape specimens classified as normal, borderline, or mild dyskaryosis, adjustment of the hc2 cutoff point resulted in similar positivity rates for both methods. These results suggest that the clinical performances (i.e., clinical sensitivity and specificity for ≥CIN3) of both methods can be compatible, which is of importance when viral typing by GP5+/6+ PCR is envisaged as a follow-up test for hc2-RCS-positive women.

MATERIALS AND METHODS

Study population, collection of cervical samples, and cervical cytology. The study, initiated as an extension of the POBASCAM (population-based cervical screening trial Amsterdam) (5), started in April 2003, and the intake finished in April 2004. Women were recruited from the national screening program via the 242 general practitioners participating in the POBASCAM trial (5). POBASCAM was initiated to compare the efficacy of HPV testing in conjunction with cytology to that of sole classical cytology. The two-armed trial was carried out within the setting of the regular Dutch nationwide cervical screening program, in which women between 30 and 60 years old are invited with screening intervals of 5 years. Further details about POBASCAM have been described previously (5). Informed consent was obtained from all participating women. For this study, we collected scrape specimens from 10,051 women participating in an extension of the POBASCAM trial. We excluded women who had a history of abnormal cytology or CIN disease within the preceding 2 years. As a consequence, of the 10,051 women recruited, 8,132 were ultimately enrolled.

Cervical smears were taken using a Cervex brush or a cytobrush. After a conventional smear was made for cytological examination, the brush was placed in a vial containing 5 ml universal collection medium (UCM) (Digene Corporation) for hrHPV testing. Upon arrival in the testing laboratory, cervical samples were concentrated to 1 ml UCM by centrifugation of vials for 10 min at 4,000 × g, and 4 ml UCM supernatant was discarded. The pellet was resuspended in 1 ml UCM and stored at −80°C until use.

Cytomorphological analysis was performed according to the CISOE-A classification, which can be translated easily as follows: 1 were initially scored hc2 positive.

A total of 232 samples had discrepant test results in relation to cytologic and histologic parameters, the McNemar test was used. The agreement was determined using the kappa value. To determine whether differences in performance of hc2-RCS and GP5+/6+ PCR and their relation to cytologic and histologic parameters, the McNemar test was used. The agreement was determined using the kappa value. To determine whether differences in performance of hc2-RCS and GP5+/6+ PCR and their relation to cytologic and histologic parameters, the McNemar test was used.

The studies of the type-specific HPV E7 PCR was performed as described previously (29) for the following types: HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68). For hc2-RCS testing, 500 µl of UCM sample material was mixed with 75 µl of guanidine-hydrochloride (8 M) and 250 µl denaturation reagent with indicator dye, briefly shaken, and denatured for 45 min at 65°C. The samples were first processed on the RSC according to the recommendations of the manufacturer (Digene Corporation). Ultimately, of each denatured sample, 75 µl was used for testing. Samples with relative light unit per cutoff value (RLU/C0) of >1 were initially scored hc2 positive.

HPV PCR testing. Sample material was prepared for GP5+/6+ PCR testing as follows: 150 µl UCM sample material was centrifuged for 10 min at 4,000 × g, and the pellet was resuspended in 1 ml Tris- HCl (pH 8.0). DNA was released after “freezing and boiling” of this 1-ml sample, and subsequently, 10 µl of this sample material was used as input in the PCR. The GP5+/6+ PCR and subsequent EIA readout system using a probe cocktail of 14 hrHPV types (i.e., HPV18, -16, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68) were performed essentially as described previously (27). The cutoff value of the C/O ratio HPV type-specific PCR-EIA was calculated as three times the average EIA value of four negative blanks that are included in each PCR run. Reverse line blot was used to genotype HPV-positive samples, which can detect up to 27 additional HPV types besides the above-mentioned 14 hrHPV types (27).

Table 1. Comparison of HR HPV positivity of hc2-RCS and GP5+/6+ PCR

<table>
<thead>
<tr>
<th>No. of specimens tested</th>
<th>Positive Positive</th>
<th>Negative Negative</th>
<th>Total Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>358</td>
<td>197</td>
<td>555</td>
</tr>
<tr>
<td>Negative</td>
<td>35</td>
<td>7,542</td>
<td>7,577</td>
</tr>
<tr>
<td>Total</td>
<td>393</td>
<td>7,739</td>
<td>8,132</td>
</tr>
</tbody>
</table>

RESULTS

Hc2-RCS and GP5+/6+ PCR test results in relation to cytology. A total of 8,132 cervical scrape specimens were analyzed by two hrHPV detection methods, i.e., hc2-RCS and GP5+/6+ PCR-EIA. The hc2-RCS test scored 555 (6.8%) samples positive overall, whereas the GP5+/6+ PCR assay revealed positivity for 393 (4.8%) samples (Table 1). The two tests gave concordant test results for 358 positive and 7,542 negative samples, with a good overall level of agreement (kappa = 0.74). A total of 232 samples had discrepant test results between hc2-RCS and the GP5+/6+ PCR assay. The number of hc2-RCS positive but GP5+/6+ PCR-negative samples (n = 197) was significantly higher than the number of
In this cross-sectional study, histologic data could be compared. The mean time between cytology and biopsy was 2.1 months (range, 0 to 8 months).

To compare the clinical value of the hc2-RCS and GP5+/6+ PCR assays, their results were first related to the cytomorphic findings. The 8,132 smears comprised 95 (1.2%) samples classified as inadequate, 7,841 (96.4%) as normal, 116 (1.4%) as borderline dyskaryosis, 20 (0.2%) as mild dyskaryosis, 28 (0.3%) as moderate dyskaryosis, 22 (0.3%) as severe dyskaryosis, and 10 (0.1%) suspected of carcinoma in situ. For further analysis, the adequate smears were categorized into two groups, i.e., normal, borderline, or mild dyskaryosis and moderate dyskaryosis or worse. Hc2-RCS and GP5+/6+ PCR test results in relation to these cytologic categories are given in Table 2. Only in women with normal, borderline, or mild dyskaryosis in an ROC curve, and these rates were compared to that of the GP5+/6+ PCR (Fig. 1). An increase of the hc2-RCS threshold to 3.2 RLU/CO resulted in positivity and negativity rates similar to those of the GP5+/6+ PCR. This value did not differ meaningfully when different cytological categories (i.e., normal cytology versus borderline dyskaryosis or worse and normal or borderline dyskaryosis versus mild dyskaryosis or worse) were used for ROC analysis (data not shown).

Hc2-RCS and GP5+/6+ PCR test results in relation to histology. In this cross-sectional study, histologic data could be collected only from women with moderate dyskaryosis or worse, since these women were directly referred for colposcopy. Data from colposcopy-directed biopsy were available from 48 women, 46 of whom had an underlying CIN lesion. The latter comprised 1 CIN1 lesion, 13 CIN2 lesions, and 32 CIN3 lesions. The mean time interval between cytological diagnosis and biopsy was 2.1 months (range, 0 to 8 months). GP5+/6+ PCR-EIA and hc2-RCS positivity rates in relation to histology are summarized in Table 3. Hc2-RCS scored 31 of 32 ≥CIN3 cases positive and GP5+/6+ PCR two fewer cases, resulting in sensitivities for ≥CIN3 of 97% (95% confidence interval, 93.8 to 100%) and 91% (95% confidence interval, 85.1 to 95.8), respectively. However, this difference was not statistically significant (P = 0.25).

When the hc2-RCS threshold value was arbitrarily increased to 2.0 RLU/CO, the number of positive scrape specimens for women with ≥CIN3 remained the same as at 1.0 RLU/CO.

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### TABLE 2. GP5+/6+ PCR and hc2-RCS results in relation to cytology

<table>
<thead>
<tr>
<th>Cytology result</th>
<th>Positive by GP and hc2-RCS</th>
<th>Negative by GP and hc2-RCS</th>
<th>Positive by GP but negative by hc2-RCS</th>
<th>Negative by GP by positive by hc2-RCS</th>
<th>Total no. of specimens</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, borderline, or mild dyskaryosis or worse</td>
<td>301</td>
<td>7,453</td>
<td>33</td>
<td>190</td>
<td>7,977</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Moderate dyskaryosis or worse</td>
<td>52</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>60</td>
<td>0.38</td>
</tr>
<tr>
<td>Total</td>
<td>353</td>
<td>7,456</td>
<td>34</td>
<td>194</td>
<td>8,037</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>

* GP, GP5+/6+ PCR.

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### TABLE 3. GP5+/6+ PCR and hc2-RCS results in relation to histology

<table>
<thead>
<tr>
<th>Intake (n*)</th>
<th>No. (%) of GP5+/6+ PCR-positive specimens</th>
<th>No. (%) of hc2-RCS-positive specimens</th>
<th>P valuec</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CIN (2)</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>—d</td>
</tr>
<tr>
<td>CIN 1 (1)</td>
<td>1 (100)</td>
<td>1 (100)</td>
<td>—</td>
</tr>
<tr>
<td>CIN 2 (13)</td>
<td>12 (92)</td>
<td>12 (92)</td>
<td>1.00</td>
</tr>
<tr>
<td>CIN 3 (32)</td>
<td>29 (91)</td>
<td>31 (97)</td>
<td>0.25</td>
</tr>
<tr>
<td>Total (48)</td>
<td>43 (90)</td>
<td>45 (94)</td>
<td>0.73</td>
</tr>
</tbody>
</table>

* Intake (n*)

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*a Mean time between cytology and biopsy: 2.1 months (range, 0 to 8 months).

*b n, number of samples.

*c McNemar.

*d No discrepant cases; not possible to determine P value.
whereas a significant reduction in test positivity was obtained for women with normal, borderline, or mild dyskaryosis (i.e., 4.7% versus 6.3% at 1.0 RLU/CO; McNemar; P < 0.0005) (Table 4). Nevertheless, at an RLU/CO value of 2.0, hc2-RCS positivity among women with normal, borderline, or mild dyskaryosis was still significantly higher than that with GP5+/6+ PCR (McNemar; P = 0.02). With use of an RLU/CO value of 3.0, the hc2-RCS positivity rate for women with normal, borderline, or mild dyskaryosis was no longer significantly different from that of the GP5+/6+ PCR (4.2% versus 4.3%; McNemar, P = 0.02). With both tests scored the same number of women with ≥CIN3 positive.

Analysis of hc2-RCS positive, GP5+/6+ PCR-negative samples. It is unlikely that the extra positivity scored by hc2-RCS with smears read as normal, borderline, or mild dyskaryosis is owing to low copy numbers of hrHPV that fall below the detection limit of the PCR assay, since the analytical sensitivity of the GP5+/6+ PCR is higher than that of hc2-RCS. To support this notion, we determined the HPV16 load by real-time PCR for 23 samples harboring a single HPV16 infection as determined by GP5+/6+ PCR followed by reverse line blot genotyping. One subset of these samples had tested negative with the hc2-RCS test (n = 11), and the other subset had tested positive with the hc2-RCS test (n = 12). Indeed, the HPV16 DNA load in the hc2-RCS negative group (median, 1.0 × 10^5; range, 1.3 × 10^4 to 1.5 × 10^5 copies/scrape specimen) was significantly lower (ANOVA, P < 0.0005) than the HPV16 load in the hc2-RCS positive group (median, 4.9 × 10^6; range, 1.0 × 10^5 to 8.7 × 10^7 copies/scrape specimen). These numbers were not influenced after stratification for cytology.

To further explain the occurrence of hc2-RCS-positive, GP5+/6+ PCR-negative cases, we addressed the influence of other potential possibilities: (i) false-negative GP5+/6+ PCR result owing to (a) inadequate crude samples for PCR due to the presence of PCR inhibitors or (b) viral integration events disrupting the GP5+/6+ PCR primer binding region in L1; (ii) false-positive hc2-RCS results owing to cross-reactivity of the hc2-RCS with other HPV types.

In order to address the first possibility, all 197 GP5+/6+ PCR-EIA negative, hc2-RCS-positive samples were retested by β-globin PCR. Only 11 (5.6%) of these samples tested negative and therefore can be considered invalid for PCR. Of the three CIN3 cases that tested negative by GP5+/6+ PCR, one case, which was also negative by hc2-RCS, was inadequate for PCR testing on the crude extract due to inhibition of the PCR. This sample, however, revealed HPV16 positivity by GP5+/6+ PCR after DNA extraction.

To determine possible integration of the HPV genome in the GP5+/6+ primer region, type-specific E7 PCR was performed on the crude extracts of the other two GP5+/6+ PCR-negative ≥CIN3 cases that were hc2-RCS positive. Both tested positive by E7 PCR and are likely to contain integrated virus with disrupted L1. These included one case containing HPV16 and one case with HPV18.

Of the remaining 186 samples that were β-globin PCR positive, the GP5+/6+ PCR was repeated, and PCR products were subjected to overall HPV typing by reverse line blotting to determine the level of cross-reactivity of the hc2-RCS test. A total of 35 (17.8%) samples were found to contain HPV types not present in the hc2-RCS probe (Table 5). Most of these cases fell in the category of normal cytology. The level of cross-reactivity of GP5+/6+ PCR could not be determined in this study, since hc2-RCS is unable to determine the HPV genotype of an infection.

### TABLE 4. Positivity rates of GP5+/6+ PCR and hc2-RCS at different RLU/CO thresholds in relation to cytology and histologically confirmed ≥CIN3

<table>
<thead>
<tr>
<th>Assay</th>
<th>Normal, borderline, or mild dyskaryosis</th>
<th>Moderate dyskaryosis or worse</th>
<th>≥CIN3</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP5+/6+ PCR-EIA</td>
<td>340 (4.3)</td>
<td>54 (90)</td>
<td>29 (91)</td>
</tr>
<tr>
<td>Hc2-RCS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RLU/CO 1.0</td>
<td>499 (6.3)†</td>
<td>56 (93)†</td>
<td>31 (97)†</td>
</tr>
<tr>
<td>RLU/CO 2.0</td>
<td>371 (4.7)†</td>
<td>56 (93)†</td>
<td>31 (97)†</td>
</tr>
<tr>
<td>RLU/CO 3.0</td>
<td>333 (4.2)†</td>
<td>54 (90)†</td>
<td>29 (91)†</td>
</tr>
</tbody>
</table>

* For normal, borderline, or mild dyskaryosis, n = 7,977; for moderate dyskaryosis or worse, n = 15; for ≥CIN3, n = 32.

* P < 0.0005 compared to positivity rate of GP5+/6+ PCR (McNemar).

P = 0.02 compared to positivity rate of GP5+/6+ PCR (McNemar); P < 0.0005 compared to positivity rate at RLU/CO 1.0 (McNemar).

No significant difference in positivity rate compared to that of GP5+/6+ PCR (McNemar, P = 0.76); P < 0.0005 compared to positivity rate at RLU/CO 1.0 (McNemar).

No significant difference in positivity rate compared to that of GP5+/6+ PCR (McNemar).

### TABLE 5. Potential cross-reactivities of the hc2-RCS probe with other HPV types

<table>
<thead>
<tr>
<th>HPV type(s)</th>
<th>No. of specimens containing type</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td></td>
</tr>
<tr>
<td>53</td>
<td></td>
</tr>
<tr>
<td>54, 70, 81, LR*</td>
<td></td>
</tr>
<tr>
<td>67</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td></td>
</tr>
<tr>
<td>70, 39</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td></td>
</tr>
<tr>
<td>82</td>
<td></td>
</tr>
<tr>
<td>LR*</td>
<td></td>
</tr>
</tbody>
</table>

Total: 35

* LR, low-risk HPV, not further specified.

In order to address the first possibility, all 197 GP5+/6+ PCR-EIA negative, hc2-RCS-positive samples were retested by β-globin PCR. Only 11 (5.6%) of these samples tested negative and therefore can be considered invalid for PCR. Of the three CIN3 cases that tested negative by GP5+/6+ PCR, one case, which was also negative by hc2-RCS, was inadequate for PCR testing on the crude extract due to inhibition of the PCR. This sample, however, revealed HPV16 positivity by GP5+/6+ PCR after DNA extraction.

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Of the remaining 186 samples that were β-globin PCR positive, the GP5+/6+ PCR was repeated, and PCR products were subjected to overall HPV typing by reverse line blotting to determine the level of cross-reactivity of the hc2-RCS test. A total of 35 (17.8%) samples were found to contain HPV types not present in the hc2-RCS probe (Table 5). Most of these cases fell in the category of normal cytology. The level of cross-reactivity of GP5+/6+ PCR could not be determined in this study, since hc2-RCS is unable to determine the HPV genotype of an infection.

### Analysis of GP5+/6+ PCR-positive, hc2-RCS-negative samples. Of the 35 GP5+/6+ PCR-positive, hc2-RCS-negative samples, 32 (91%) harbored an HPV type that is present in the hc2-RCS probe (Table 6). HPV types that were most often missed by hc2-RCS were HPV16, HPV45, HPV56, and HPV31. Most of these cases (28/32) were cytologically normal or borderline dyskaryotic.

In the 23 samples used for HPV 16 load analysis by real-time PCR (as detailed above), we also determined the amount of cells per scrape by β-globin gene real-time PCR. Interestingly, the amount of cells per scrape in the hc2-RCS positive smears (median, 4.0 × 10^6; range, 2.8 × 10^5 to 2.3 × 10^7 cells/scrape) was significantly higher than that found in the hc2-RCS negative group (median, 1.2 × 10^6; range, 1.5 × 10^5 to 8.6 × 10^6 cells/scrape; ANOVA, P = 0.04). The smaller amount of DNA

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in these smears may have contributed to the negative test results of the hc2-RCS. However, clinical follow-up data need to be gathered before conclusions can be made as to what extent these cases reflect clinically relevant infections.

**DISCUSSION**

In this study we compared the performances of the hc2-RCS test and the hrHPV GP5+/6+ PCR-EIA in relation to cytologic and histologic parameters of women participating in a population-based screening trial. The overall agreement between both tests was good and was comparable to that found by Kulmala et al. (2004) between the manual hc2 and GP5+/6+ PCR assays (i.e., kappa 0.67) (16).

Differences in performances of hc2-RCS and GP5+/6+ PCR could mainly be explained by differences in assay thresholds. Still, the higher positivity rate of the hc2-RCS at an RLU/CO value of 1.0 compared to that of the GP5+/6+ PCR in our study is somewhat surprising, given the lower analytical sensitivity of the hc2 assay. The latter was supported by higher viral load values in HPV16 PCR-positive samples that were also hc2/RCS positive than in those that were hc2-RCS negative. However, we collected evidence that part of the hc2-RCS positivity in GP5+/6+ PCR-negative samples can be attributed to some cross-reactivity of the hc2-RCS assay with HPV types that are not covered by the probes of the hc2-RCS test. Similar results have been obtained in other studies (6, 12). A much smaller proportion of hc2-RCS-positive/GP5+/6+ PCR-negative samples could be explained by PCR failures due to PCR inhibitors in the crude extracts, while the presence of integrated viral DNA with a disrupted GP5+/6+ region is likely to be a rare event that only occasionally may occur in high-grade lesions. A major part of hc2-RCS-positive/GP5+/6+ PCR-negative test results, which mainly involved scrapings from women with normal, borderline, or mild dyskaryosis and weakly positive hc2/RCS values, is likely to reflect a certain level of background noise when the hc2-RCS assay is applied at an RLU/CO value of 1.0. However, our data indicate that a more optimal signal-to-noise ratio of the hc2-RCS method can be obtained by adjusting the threshold of this assay to levels at which the results of this test better match those of GP5+/6+ PCR. Moreover, since an interlaboratory reproducibility evaluation by Castle et al. (2004) revealed that the reproducibility of an hc2-positive test is lowest for women with normal cytology, particularly those with RLU/CO values between 1 and 3, adjustment of the hc2 thresholds is likely to also increase the reproducibility of the assay (8).

Our results seem to contradict the findings of Kulmala et al. (2004), who detected a higher positivity rate with GP5+/6+ PCR than with the manual hc2 assay (i.e., 33.8% and 27.9%, respectively). This difference may be explained by the fact that their study population generally involved younger women displaying a much higher HPV prevalence rate that may have masked the potential hc2 noise. On the other hand, the overall hc2-RCS positivity rate (i.e., 6.8%) in our study is in the same range as that obtained with the manualhc2 in the HART study (i.e., 7.6%), which involved women of a population-based screening cohort with a similar age distribution (10).

In this study a threshold of 2.0 RLU/CO for the hc2-RCS test would result in an increased clinical specificity for women with ≥CIN3, while no additional lesions ≥CIN3 were missed. Therefore, in case the hc2 is used as a primary screening tool, we, like others (7, 10), feel that the assay threshold easily can be increased to 2.0 RLU/CO, since this would result in a higher specificity, thereby minimizing the unnecessary follow-up of women with transient infection. Alternatively, when genotyping by GP5+/6+ PCR is envisaged as a reflex test for hc2-RCS-positive women without cytological abnormalities, adaptation of the hc2-RCS cutoff is less crucial, since in that scenario, the cutoff of the GP5+/6+ PCR assay would be decisive for further management. Still, it should be realized that definitive figures about clinical sensitivity and specificity for ≥CIN3 (24) and consequently the clinically most informative RLU/CO threshold can be calculated only when all follow-up information on women with normal, borderline, or mild dyskaryosis has been gathered.

Conversely, Ordi et al. (19) advocated the use of 1.0 RLU/CO, since their data showed that increasing the cutoff level would not lead to an increase in specificity. However, the women in their study were selected on the basis of cytological abnormality (ASC-US or worse). For such a high-risk population, specificity is rather low regardless of which cutoff level is used. We even feel that in case the hc2-RCS cutoff is increased to 2.0 or 3.0 RLU/CO, the clinical specificity of hrHPV testing for ≥CIN3 still needs improvement, since a substantial number of women that score hc2 positive at these cutoff values do not have or develop ≥CIN3. This may be achieved by further stratifying HPV-positive women by genotyping (4), viral load assessment (11, 28) and/or analysis of the presence of E6/E7 mRNA (9).

Most importantly, when sensitivity and specificity to detect lesions ≥CIN3 are considered equally important aspects of overall accuracy, both assays studied herein are similarly accurate, provided that the hc2-RCS cutoff is slightly adapted. For primary and secondary screening, this opens possibilities for a combination test of hc2-RCS and GP5+/6+ PCR, in which the hc2-RCS test, being easy and robust and therefore an ideal tool for application in large screening programs, is used first and the GP5+/6+ PCR as a reflex test (i.e., testing only of hc2-RCS-positive samples) for genotyping of hc2-RCS-positive women. The latter assay requires special skills and a more stringent infrastructure to reduce PCR-related contamination.
COMPARISON OF hc2 ASSAY and GP5+/+6+ PCR METHOD


Chapter 10

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
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

N W J Bulkmans, M van Ballegooijen, P J F Snijders, C J L M Meijer

Summary
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 (≥6·5 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=0·007). 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=0·001). 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. 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. 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, 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. Moreover, variability of HPV DNA testing, both between and within laboratories, is lower than that of cytological testing. 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. 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. 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 17 155 of the 44 938 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. 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 cyotechnicians 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) 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). 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 for cervical screening in the Netherlands. 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.24 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.25 Validation of this assay and inter-method and intra-method comparisons have been described previously.26 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.27,28 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.29,30 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 CIN1+ 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.31

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 χ² 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

<table>
<thead>
<tr>
<th>Interventions</th>
<th>Tested for HPV DNA</th>
<th>HPV DNA positive*</th>
<th>Control group</th>
<th>Tested for HPV DNA</th>
<th>HPV DNA positive*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inadequate</td>
<td>9 (0.1%)</td>
<td>0 (0%)</td>
<td></td>
<td>12 (0.1%)</td>
<td>1 (0%)</td>
</tr>
<tr>
<td>Normal</td>
<td>8231 (97.2%)</td>
<td>8260 (99.9%)</td>
<td>8230 (97.2%)</td>
<td>8261 (99.9%)</td>
<td>779 (94.9%)</td>
</tr>
<tr>
<td>Borderline or mild dyskaryosis</td>
<td>379 (2.3%)</td>
<td>324 (86.5%)</td>
<td>384 (97.1%)</td>
<td>324 (86.5%)</td>
<td>314 (90.7%)</td>
</tr>
<tr>
<td>Moderate dyskaryosis or worse</td>
<td>56 (0.7%)</td>
<td>48 (85.7%)</td>
<td>56 (100%)</td>
<td>56 (100%)</td>
<td>56 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>8575 (100%)</td>
<td>8599 (99.5%)</td>
<td>8580 (100%)</td>
<td>8598 (99.5%)</td>
<td>8598 (99.5%)</td>
</tr>
</tbody>
</table>

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.

www.thelancet.com Published online October 4, 2007 DOI:10.1016/S0140-6736(07)61450-0
that showed borderline or mild dyskaryosis. Overall, a sample size of 18,000 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).

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, 44,938 women were randomly assigned to the intervention or control group. By February, 2007, 18,403 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 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.

Table 1: Cytological and HPV DNA test results at subsequent round

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Tested for HPV DNA</th>
<th>HPV DNA positive*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inadequate</strong></td>
<td>24 (0·3%)</td>
<td>14</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>Normal</strong></td>
<td>6697 (97·2%)</td>
<td>4421</td>
<td>109 (2·5%)</td>
</tr>
<tr>
<td><strong>Borderline or mild dyskaryosis</strong></td>
<td>218 (1·9%)</td>
<td>94</td>
<td>37 (38·7%)</td>
</tr>
<tr>
<td><strong>Moderate dyskaryosis or worse</strong></td>
<td>38 (0·6%)</td>
<td>18</td>
<td>14 (77·8%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>6887 (100%)</td>
<td>4547</td>
<td>150 (3·3%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Intervention group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CIN2</strong></td>
<td>30 (0·3%)</td>
<td>23 (0·2%)</td>
</tr>
<tr>
<td><strong>CIN3</strong></td>
<td>60 (0·7%)</td>
<td>37 (0·4%)</td>
</tr>
<tr>
<td><strong>ACIS</strong></td>
<td>3 (0·4%)</td>
<td>1 (0·1%)</td>
</tr>
<tr>
<td><strong>AdCa</strong></td>
<td>4 (0·5%)</td>
<td>2 (0·3%)</td>
</tr>
<tr>
<td><strong>SCC</strong></td>
<td>1 (0·2%)</td>
<td>1 (0·1%)</td>
</tr>
<tr>
<td><strong>CIN3+</strong></td>
<td>6 (0·7%)</td>
<td>5 (0·6%)</td>
</tr>
<tr>
<td><strong>CIN2+</strong></td>
<td>68 (1·1%)</td>
<td>73 (0·8%)</td>
</tr>
</tbody>
</table>

Data are n or n (%). 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

<table>
<thead>
<tr>
<th>Time (years)</th>
<th>Number of lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C</td>
</tr>
<tr>
<td>2</td>
<td>C</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
</tr>
<tr>
<td>5</td>
<td>C</td>
</tr>
<tr>
<td>6</td>
<td>C</td>
</tr>
<tr>
<td>7</td>
<td>C</td>
</tr>
<tr>
<td>8</td>
<td>C</td>
</tr>
</tbody>
</table>

Figure 2: Year of diagnosis

I=intervention. C=control.

www.thelancet.com Published online October 4, 2007 DOI:10.1016/S0140-6736(07)61450-0
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% (9031/13 725) 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 rounds 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 83% (95% CI 62–97) in the intervention group and 83% (70–93) in the control group.

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

<table>
<thead>
<tr>
<th></th>
<th>Intervention group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline cytological and HPV DNA testing</td>
<td>Baseline cytological and HPV DNA testing</td>
</tr>
<tr>
<td></td>
<td>HPV DNA positive</td>
<td>HPV DNA negative</td>
</tr>
<tr>
<td></td>
<td>Normal Borderline or mild dyskaryosis Moderate dyskaryosis or worse Total</td>
<td>Normal Borderline or mild dyskaryosis Moderate dyskaryosis or worse Total</td>
</tr>
<tr>
<td>Baseline round</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN3+ detected</td>
<td>13</td>
<td>2*</td>
</tr>
<tr>
<td>CIN2+ detected</td>
<td>25</td>
<td>4*</td>
</tr>
<tr>
<td>Subsequent round</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN3+ detected</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>After normal cytology/HPV DNA positive</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Borderline dyskaryosis or worse/HPV DNA positive</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Borderline dyskaryosis or worse/HPV DNA negative</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Borderline dyskaryosis or worse/no HPV DNA result</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Other†</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>CIN3+ detected</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>After normal cytology/HPV DNA positive</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Borderline dyskaryosis or worse/HPV DNA positive</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Borderline dyskaryosis or worse/HPV DNA negative</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Borderline dyskaryosis or worse/no HPV DNA result</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Other†</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

*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.
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

<table>
<thead>
<tr>
<th>CIN3+</th>
<th>CIN2+</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td><strong>Risk</strong></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Crude</strong></td>
<td><strong>Adjusted</strong>*</td>
</tr>
<tr>
<td><em>Intervention group</em></td>
<td></td>
</tr>
<tr>
<td>Normal cytological results and HPV DNA negative (n=7980)</td>
<td>22</td>
</tr>
<tr>
<td>HPV DNA negative (n=8113)</td>
<td>29</td>
</tr>
<tr>
<td><em>Control group</em></td>
<td></td>
</tr>
<tr>
<td>Normal cytological results (n=8330)</td>
<td>70</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Referral</th>
<th>Biopsy</th>
<th>CIN3+</th>
<th>CIN2+</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number per woman screened</strong></td>
<td><strong>Rate per woman screened</strong></td>
<td><strong>Number per woman referred</strong></td>
<td><strong>Rate per woman referred</strong></td>
</tr>
<tr>
<td><em>Intervention group</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline round (n=8575)</td>
<td>201</td>
<td>2·3% (2·0–2·7)</td>
<td>136</td>
</tr>
<tr>
<td>Subsequent round (n=6887)***</td>
<td>87</td>
<td>1·3% (1·0–1·6)</td>
<td>59</td>
</tr>
<tr>
<td><em>Control group</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline round (n=8580)</td>
<td>115</td>
<td>1·3% (1·1–1·6)</td>
<td>83</td>
</tr>
<tr>
<td>Subsequent round (n=6838)***</td>
<td>129</td>
<td>1·9% (1·6–2·2)†</td>
<td>90</td>
</tr>
</tbody>
</table>

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
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. 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, 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%. 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%. Other similar trials (eg, the Finnish Randomised Public Health Trial, ARTISTIC in the UK, SWEDESCREEN in Sweden, CCCaST in Canada) 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%; others with Hybrid Capture 2 testing reported figures of between 6-1% and 10-7%. Previous population-based screening studies with Hybrid Capture 2 reported figures of 6–4–8.1%. 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. These assays have a similar sensitivity and specificity when used for the detection of CIN2+. 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. Therefore, HPV DNA test requirements should be incorporated into the guidelines of the screening programme.

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. 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). 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\textsuperscript{\text{1-3,5-9}} 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 HPV DNA 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.\textsuperscript{9} 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,\textsuperscript{10-12} 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.

Conflicts of interest statement

CJLM is supervised 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.

Acknowledgments

The study was funded by ZON, Zorg Onderzoek Nederland (Netherlands Organisation for Health Research and Development; grant 30-05220). We gratefully acknowledge the work of the 242 general practitioners and their assistants, the Municipal Health Service Southwest of Amsterdam, Medial, and DHV Kennemerland-Haarlemmermeer e.o. and PALGA. We especially thank research staff and technicians of the Unit Molecular Pathology, VU University Medical Centre, Amsterdam for HPV DNA testing, the cytotechnologists for cytological testing and logistics, and the administrative co-workers and the information technology team of the Department of Pathology, VU University Medical Centre, Amsterdam, for their supportive work. We would like to dedicate this manuscript to Jan M M Walbooms, who passed away on Feb 2, 2000, and who was a devoted initiator of the POBASCAM trial.

References


Chapter 11

Summary and General discussion

1. HrHPV testing detects clinically relevant ≥CIN2 lesions

2. HrHPV and attendance rate

3. The hrHPV test to be used in primary cervical screening

4. Risk management and hrHPV genotyping

5. Future developments: HPV vaccination

6. Conclusion
11. General discussion

Here we will discuss our findings as presented in the preceding chapters in relation to possible implementation of hrHPV testing in primary cervical screening.

11.1 HrHPV testing detects clinically relevant ≥CIN2 lesions

In 1997, at the time the POBASCAM trial was initiated, we knew from relatively small pilot studies that hrHPV testing had a higher sensitivity and negative predictive value for the detection of ≥CIN2 lesions than cytology, at the cost of a slightly lower specificity (1). This result has been confirmed by others and by us (Chapter 2) (2-6).

It was however, still unknown whether the additionally detected lesions by hrHPV testing were clinically relevant, i.e., whether it concerned non-regressing lesions. Several randomised controlled trials involving thousands of women are presently still ongoing with the objective to answer this question and to assess the long-term impact of hrHPV testing. These trials assess the effectiveness of primary screening by only hrHPV testing or by hrHPV testing in combination with cytology compared to screening by conventional or liquid-based cytology (Finland-Finnish Randomised Public Health Trial N=14,149, age 30-60 years; Italy-NTCC, N=33,364, age 35-60 years; UK-ARTISTIC N=19,344, age 30-64 years; Sweden-SWEDESCREEn N=12,527, age 32-38 years; Canada-CCCaST Canada, N=9,667, age 30-69 years; The Netherlands-POBASCAM N=49,220, age 29-61 years; The Netherlands-VUSA-screen N=50,000, age 30-60 years (7-13)).

In our publication concerning five years follow-up over two screening rounds of 17,155 women in the POBASCAM trial (Chapter 10) our data indicate that the earlier detected lesions by combined hrHPV and cytology screening are not a subset of regressive lesions but indeed clinically relevant lesions. This assumption is based on the notion that over two screening rounds, both in the intervention and control group of the POBASCAM trial the same number of ≥CIN2 lesions were detected. However, in the intervention group (women screened by hrHPV testing and cytology), many lesions were detected earlier, i.e., in the first screening round 56% more ≥CIN2 lesions compared to only cytology screening, and in the second round 47% less ≥CIN2 lesions. A preliminary cost-effectiveness analyses indicates that this earlier detection of ≥CIN2 leads to a larger reduction in the incidence and mortality of cervical cancer than cytology (14). The 5-years cumulative risk of ≥CIN2 after a combined negative hrHPV and cytology test result was reduced by 64%, compared to the risk after normal cytology only. These results could permit an extension of the screening interval by at least 1 year. Since the risk after a negative hrHPV test result only was similar to the risk after a combined negative test result, the cost-effectiveness of adding cytological testing to hrHPV test is thus doubtful. After the 5-year follow-up data of the POBASCAM trial have been published, the greater sensitivity for the detection of ≥CIN2 by hrHPV testing as compared with cytology has been confirmed in population-based trial data by the Canadian trial (15) and our results concerning the detection of clinically relevant ≥CIN2 lesions have been confirmed by the Swedish trial (16).

Hence, screening by combined hrHPV testing and cytology results in an earlier detection of clinically relevant ≥CIN2 lesions. Earlier detection of such lesions and a strongly reduced five-year risk of ≥CIN2 could permit an extension of the screening interval.
11.2 HrHPV and attendance rate

We have shown that adding hrHPV testing to cervical screening does not decrease participation rates (Chapter 4), provided that 1) the general practitioners and other healthcare providers are well trained, and 2) the women concerned are informed regarding hrHPV. In the POBASCAM trial the training consisted of postgraduate courses for the general practitioners on hrHPV and its relationship with cervical cancer, and a leaflet for women invited to cervical screening with information on the nature of hrHPV infections, the lifetime prevalence, the clearance rate, and the increased risk for cervical cancer.

Before initiating the POBASCAM trial, a survey conducted among 1551 Dutch women indicated that hrHPV testing would not interfere with participation in cervical screening (17). Thus, the concern about the influence of hrHPV testing on the attendance rate in cervical screening because of the perceived association of cervical cancer with sexually transmitted infections (18;19) has been refuted by the attendance of the women in the POBASCAM trial. Furthermore, it has been shown that 34.2% of the women not attending cervical screening by cytology who were offered a self-sampling device for hrHPV testing did respond, thereby leading to a true increase in the attendance rate, which could not be obtained by a repeated invitation for cytology screening (20).

Hence, our experience is that implementation of hrHPV testing to the regular screening programme is well accepted, without a decrease in participation rate if attention is paid to the nature of information regarding hrHPV given to the women to be screened and especially to general practitioners and other health care providers.

11.3 The hrHPV test to be used in primary cervical screening

Which hrHPV test should be used in case of the implementing hrHPV testing in cervical screening? Currently, there are only two tests that have been extensively analysed for their performance and can be considered clinically validated, i.e., the GP5+/6+ PCR and Hybrid Capture 2 (HC2) assays (6;21). These assays have a similar sensitivity and specificity when used for the detection of ≥CIN2 lesions (Chapter 9). The HC2 rapid capture system (RCS) allows high-throughput population screening in an automated format. The GP5+/6+ PCR-EIA assay has the advantage that direct genotyping is possible on the hrHPV-specific PCR products(22). Both HC2 and GP5+/6+ PCR show good to excellent intra- and inter-laboratory reproducibility (23-25).

The hrHPV test to be used in screening should display a good balance between sensitivity and specificity for the detection of ≥CIN2. Especially in cervical screening, tests with a lower specificity for ≥CIN2 lesions in favour of a very high sensitivity for hrHPV should not be used (25-27). Therefore, it is essential that hrHPV test requirements should be incorporated into the guidelines of the screening programme (25;28).

Recently in The Netherlands, the general requirements for an hrHPV test to be used in screening have been formulated, including a good sensitivity for ≥CIN2, a high intra- and inter-laboratory reproducibility, and clinical validation (24).

As mentioned above, the GP5+/6+ PCR-EIA and the Hybrid Capture 2 tests are the only two clinically validated hrHPV tests that currently suit the general requirements for usage in cervical screening.
11.4 Risk management and HPV genotyping

Women with a prevalent hrHPV infection have an increased risk of cervical carcinoma. The odds ratio for cervical cancer associated with the presence of any hrHPV type was even 158.2 (95%CI 113.4 to 220.6) in a pooled analysis of 11 case-control studies conducted by the IARC in 11 countries involving 5000 women (21).

A full cost-effective analysis is essential to determine whether primary hrHPV testing alone is the preferred strategy for cervical screening. The following screening algorithm is one of the possibilities for the risk management of hrHPV positive women. With primary cervical screening by hrHPV testing we expect about 5% of the women aged 30-60 years to have a prevalent hrHPV infection (Chapter 3). Since cytology is a very good tool for risk stratification among hrHPV positive women, hrHPV-positive samples should be subjected to reflex cytology (i.e. cytology using the residual cells of the hrHPV sample). About 30% of the hrHPV-positive samples, i.e. 1.5% of the screened population, will have abnormal cytology. These women should be referred for colposcopy because of the very high risk of ≥CIN2 (risk of ≥CIN2, after BMD 33% 95%CI 28-38, after >BMD 79% 95%CI 74-83).

Because of the increased risk of developing ≥CIN2 lesions among the remainder 3.5% of women with a positive hrHPV test and normal cytology, these women should be recalled earlier than the next screening round, (5 year). We showed that repeat cytology and hrHPV testing (Chapter 7) and HPV genotyping (Chapter 5-8) can be used for risk stratification of these women with normal cytology. To illustrate the difference in risk of ≥CIN2 for different hrHPV types: women with normal cytology and HPV16/18 had a cumulative 18-month risk for ≥CIN2 of 25% whereas the risk for women infected with other hrHPV types was 5.3% (Chapter 7).

Based on the data as described in the previous chapters, cost-effectiveness analysis is presently being performed, to determine the optimal screening algorithm for cervical screening using hrHPV testing, including issues such as screening interval, starting age, and how cytology and HPV genotyping can be used for an effective follow-up of hrHPV positive women. An example of such a possible algorithm is shown in Figure 1.

![Figure 1: Possible follow-up scheme for hrHPV-positive women participating in cervical screening by primary HPV testing using cytology and hrHPV genotyping as triage tests.](https://example.com/image.png)

11.5 Future developments: HPV vaccination

Use of HPV vaccines might have an impact as a preventive strategy for cervical cancer. Two HPV vaccines, Cervarix® and Gardasil®, are commercially available in The Netherlands. These vaccines induce high titers of neutralising antibodies, preventing infection of cervical epithelial cells by HPV types present in the vaccine. The vaccines have high efficacy against HPV16/18 related ≥CIN2 in women negative for HPV16/18 DNA at the time of vaccination (29;30). Therefore vaccination before sexarche (9-14 year) is the most optimal time for vaccination. The prophylactic vaccination with only HPV16 and HPV18 has the potential to protect against ~75% of the cervical
cancer cases since 70% of the cervical carcinomas is attributed to HPV16 or HPV18, and 5% protection can result from the cross-protection against HPV31 and HPV45 (29;30). The vaccines do not have a therapeutic effect on pre-existing HPV16/18 infections, nor pre-existing CIN lesions caused by these types.

From a public-health point of view and in order to maximise the preventive effect of vaccination, a high coverage of prepubertal women should be obtained. This outcome can be achieved by incorporation of HPV vaccines into existing national vaccination programmes offered to prepubertal women. The question can be asked whether cervical screening in the era of prophylactic vaccination will still be necessary. Since prophylactic vaccination of prepubertal women potentially prevents against 75% of cervical carcinomas and many women of the present generation of adult women are not vaccinated cervical screening will still remain. It can be expected that hrHPV testing rather than cytology will be the primary screening tool in the near future (31).

11.6 Conclusion

In this thesis we present definite proof that primary cervical screening by hrHPV testing detects clinically relevant ≥CIN2 lesions earlier than cytology, and does not lead to detection of a subset of regressive CIN lesions. The earlier detection of clinically relevant CIN lesions and the 64% reduced risk for ≥CIN2 lesions after a negative hrHPV test after 5 years compared to normal cytology, could permit extension of the current screening interval. This would be advantageous to women because of the reduction in the life-time number of screening test and referrals. This may result in an increased attendance to the screening programme. Since earlier detection of ≥CIN2 leads to a reduction in person years at risk in which ≥CIN2 lesions can progress to cervical cancer, the earlier diagnoses of ≥CIN2 might lead to a reduction in cervical cancer (14;15). Provided that all parties involved in screening, especially the general practitioners, are supplied with adequate information about hrHPV, primary cervical screening by hrHPV is well accepted and does not lead to a decrease in attendance (chapter 4). HrHPV tests to be used in primary cervical screening should display an optimal balance between sensitivity and specificity for the detection of ≥CIN2 and test requirements should be incorporated in screening guidelines. Test requirements have recently been formulated by Hesselink (25). Currently, two tests, i.e., GP5+/6+ PCR EIA and HC2 are clinically validated and can be used as primary screening tools. A full cost-effective analysis is presently performed on the basis of our data to determine which algorithm for hrHPV testing should be used. In addition, cytology and HPV genotyping seem promising tests to further stratify hrHPV-positive women and model studies are ongoing to find out the most optimal, simple and women-friendly follow-up algorithm. It can be expected that in the future a molecular test will replace the triage by reflex cytology, thereby changing from a “subjective technique” to an “objective highly reproducible test”.
Reference List


Samenvatting en Algemene Discussie
In dit proefschrift worden studies gepresenteerd die tezamen beoogden de waarde van implementatie van de hoog-risico humaan papillomavirus (hrHPV) test in het bevolkingsonderzoek baarmoederhals-kanker in kaart te brengen.

Testen op hrHPV detecteert klinisch relevante ≥CIN2 laesies

In 1997, toen de POBASCAM trial (ook wel HPVBOB studie) werd geïnitieerd, was, op basis van een relatief kleine pilot studie, bekend dat het testen op hrHPV een hogere sensitiviteit en negatief voorspellende waarde had voor de detectie van baarmoederhalskanker en hoog-gradige voorloper-afwijkingen daarvan (≥CIN2 laesies) dan cytologie (1). Dit ging wel ten koste van een iets lagere specificiteit. Dit resultaat werd later bevestigd door anderen en door ons (Hoofdstuk 2) (2-6).

Het was echter nog steeds onbekend of de met een hrHPV test extra gedetecteerde ≥CIN2 laesies klinisch relevant waren, d.w.z. of het niet-regressieve laesies betrof. Meerdere gerandomiseerde studies, betreffende duizenden vrouwen zijn nog steeds gaande om deze vraag te beantwoorden en om het lange termijn effect van het testen op hrHPV vast te stellen. In deze studies wordt de effectiviteit van primaire screening door middel van het testen op hrHPV al dan niet in combinatie met cytologie, vergeleken met screening middels alleen klassieke of dunne laag cytologie. (Finland-Finnish Randomised Public Health Trial N=14,149, leeftijd 30-60 jaar; Italië-NTCC, N=33,364, 35-60 jaar; UK-ARTISTIC N=19,344, 30-64 jaar; Zweden-SWEDESCREEN N=12,527, 32-38 jaar; Canada-CCCaST Canada, N=9,667, 30-69 jaar; Nederland-POBASCAM N=49,220, 29-61 jaar; Nederland-VUSA-screen N=50,000, 30-60 leeftijd (7-13)).

In onze publicatie betreffende vijf-jaars follow-up over twee screeningsronden van 17,155 vrouwen in de POBASCAM studie (Hoofdstuk 10), laten de gegevens zien dat de eerder gedetecteerde laesies door gecombineerd hrHPV cytologie testen niet regressieve laesies betreft maar daadwerkelijk klinisch relevante laesies. Deze veronderstelling is gebaseerd op de observatie dat over twee screeningsronden, zowel in de interventie groep (vrouwen gescreeën met zowel hrHPV als cytologie test) als in de controle groep (vrouwen gescreeën met alleen cytologie) van de POBASCAM studie hetzelfde aantal ≥CIN2 laesies werd gedetecteerd. Echter, in de interventie groep, werden veel laesies eerder gedetecteerd, d.w.z. in de eerste screeningsronde 56% meer ≥CIN2 laesies vergeleken met de controle groep, en in de tweede ronde 47% minder ≥CIN2 laesies. Een voorlopige kosten-effectiviteits analyse laat zien dat het eerder detecteren van deze ≥CIN2 laesies met behulp van een hrHPV test leidt tot een grotere reductie in de incidentie en mortaliteit van baarmoederhalskanker dan het testen met alleen cytologie (14). Het cumulatieve 5-jaars risico op ≥CIN2 na een gecombineerde negatieve hrHPV en cytologie testuitslag lag 64% lager dan na alleen een negatieve cytologie uitslag. Deze resultaten geven aan dat door gebruik van een hrHPV test het screeningsinterval met tenminste 1 jaar kan worden verlengd. Omdat het risico na alleen een negatieve hrHPV test nagenoeg gelijk was aan het risico verkregen na een gecombineerd (d.w.z. hrHPV en cytologie) negatief test resultaat, is de waarde van het toevoegen van cytologie aan het testen op hrHPV twijfelachtig. Nadat de 5-jaars follow-up gegevens van de POBASCAM studie gepubliceerd waren, is de hogere sensitiviteit van de hrHPV test voor de detectie van ≥CIN2 bevestigd in een grote Canadese populatie studie (15) en is het feit dat de
hrHPV test leidt tot een vroegere detectie van klinisch relevante ≥CIN2 laesies bevestigd in een Zweedse populatie studie (16).

Samenvattend resulteert gecombineerd testen op hrHPV en cytologie binnen het bevolkingsonderzoek baarmoederhalskanker in een vroegere detectie van klinisch relevante ≥CIN2 laesies. De vroegere detectie van zulke laesies en het daarmee gepaard gaande sterk verlaagd vijf-jaars risico op ≥CIN2 maken een verlenging van het screeningsinterval mogelijk.

HrHPV en deelnamepercentage
Verder vonden we dat het toevoegen van het testen op hrHPV aan het bevolkingsonderzoek het deelnamepercentage niet vermindert (Hoofdstuk 4), vooropgesteld dat 1) de huisartsen en andere gezondheidsmedewerkers goed getraind zijn, en 2) de vrouwen goed geïnformeerd werden over hrHPV. In de POBASCAM studie bestond de training uit nascholingscursussen voor huisartsen over hrHPV en de relatie met baarmoederhalskanker. Daarnaast was een informatiefolder samengesteld voor de vrouwen die uitgenodigd werden voor hrHPV. Deze folder bevatte informatie over het natuurlijke beloop van hrHPV infecties, de lifetime prevalentie, de klaring percentages, en het verhoogde risico op baarmoederhalskanker.

Voordat de POBASCAM studie gestart werd, is een enquête verricht onder 1551 Nederlandse vrouwen die aangaf dat het testen op hrHPV niet van invloed zou zijn op het deelnamepercentage van het bevolkingsonderzoek (17). Verder is de bezorgdheid over een mogelijke negatieve invloed van het testen op hrHPV op het deelnamepercentage van het bevolkingsonderzoek vanwege de veronderstelde associatie met seksueel overdraagbare aandoeningen (18;19) ongegrond bevonden op basis van de feitelijke deelname van vrouwen aan de POBASCAM studie. Verder is het bekend dat 34.2% van de vrouwen die niet deelnemen aan het reguliere bevolkingsonderzoek met cytologie, wel reageerden wanneer een zelfafnameverzamelaar voor het zelf verzamelen van vaginaal materiaal voor het testen op hrHPV werd aangeboden. Deze mogelijkheid leidt dus tot een substantiële toename van het deelnamepercentage, wat niet bereikt kon worden met een herhalingsuitnodiging voor cytologie (20).

Kortom, implementatie van de hrHPV test in het reguliere bevolkingsonderzoek wordt goed geaccepteerd en leidt niet tot een daling van het deelnamepercentage, mits er aandacht wordt besteed aan informatie betreffende het natuurlijke beloop van een hrHPV infectie aan de vrouwen en vooral ook aan de huisartsen en andere gezondheidsmedewerkers.

De te gebruiken hrHPV test in het bevolkingsonderzoek baarmoederhalskanker
Welke hrHPV test dient gebruikt te worden in geval van implementatie van een test op hrHPV in het bevolkingsonderzoek baarmoederhalskanker? Thans zijn er slechts twee testen die uitvoerig geanalyseerd zijn en in grote studies even accuraat bleken te zijn voor de detectie van ≥CIN2 met betrekking tot hun performance en daarom als klinisch gevalideerd beschouwd kunnen worden. Dit betreffen de GP5+/6+ PCR en de Hybrid Capture 2 (HC2) test (6;21). Deze testen hebben een vergelijkbare sensitiviteit en specificiteit voor de detectie van ≥CIN2 laesies (Hoofdstuk 9). Het HC2 rapid capture systeem (RCS) is een geautomatiseerd systeem dat uitermate geschikt is voor testen op grote schaal ten behoeve van het bevolkingsonderzoek. De GP5+/6+ PCR test heeft het voordeel dat directe genotypering op het hrHPV-specifieke PCR product mogelijk is (22).
Zowel HC2 als GP5+/6+ PCR hebben een goede tot excellente intra- en inter-laboratorium reproduceerbaarheid (23-25).

De in het bevolkingsonderzoek te gebruiken hrHPV test dient een goede balans tussen sensitiviteit en specificiteit voor de detectie van ≥CIN2 laesies te hebben. Speciaal voor het bevolkingsonderzoek baarmoederhalskanker dienen testen met een lagere specificiteit voor ≥CIN2 laesies ten faveure van een zeer hoge sensitiviteit voor hrHPV niet gebruikt te worden (25-27). Daarom is het essentieel dat richtlijnen voor hrHPV test-vereisten worden opgenomen in de richtlijnen voor het bevolkingsonderzoek programma (25;28). In Nederland zijn recent zulke richtlijnen opgesteld, waarin een goede sensitiviteit en specificiteit voor ≥CIN2, een hoge intra- en inter-laboratorium reproduceerbaarheid, en klinische validatie zijn opgenomen (24).

Zoals hierboven genoemd, zijn de GP5+/6+ PCR-EIA en de HC2 testen de enige twee hrHPV testen die thans voldoen aan deze test-vereisten voor gebruik bij het bevolkingsonderzoek baarmoederhalskanker.

**Risico management en HPV genotypering**

Vrouwen met een hrHPV infectie hebben een verhoogd risico op baarmoederhalskanker. De odds ratio voor baarmoederhalskanker bij aanwezigheid van een willekeurig hrHPV type was zelfs 158,2 (95% betrouwbaarheidsinterval (BI): 113,4 tot 220,6) in een gecombineerde analyse van 11 case-control studies uitgevoerd door de IARC in 11 landen onder 5000 vrouwen (21).

Een volledige kosten-effectiviteits analyse is noodzakelijk om te bepalen of primair testen met uitsluitend hrHPV de voorkeursstrategie voor het bevolkingsonderzoek op baarmoederhalskanker is. De hieronder genoemde parameters behoren tot de mogelijkheden voor risico management van hrHPV positieve vrouwen binnen het bevolkingsonderzoek. Bij een bevolkingsonderzoek waarbij primair op hrHPV getest wordt, verwachten we dat ongeveer 5% van de vrouwen tussen de 30 en 60 jaar een prevalente infectie met hrHPV hebben (Hoofdstuk 3). Aangezien cytologie een zeer goed hulpmiddel is voor risico stratificatie bij hrHPV positieve vrouwen, zouden de hrHPV-positieve uitstrijk monsters beoordeeld moeten worden met reflex cytologie (i.e. cytologie op de overblijvende cellen van het hrHPV monster). Ongeveer 30% van de hrHPV-positieve monsters (1,5% van de gescreende vrouwen) zal afwijkende cytologie laten zien. De betrokken vrouwen dienen dan verwezen te worden voor colposcopie vanwege het zeer sterk verhoogde risico op ≥CIN2 (risico op ≥CIN2, na Pap2/3a1:33%; 95%BI 28-38, na >Pap2/3a1: 79%; 95%BI 74-83).

Vanwege het risico op het ontstaan van ≥CIN2 laesies onder de resterende 3.5% vrouwen met een positieve hrHPV test en normale cytologie, dienen deze vrouwen eerder teruggeroepen te worden dan de volgende ronde van het bevolkingsonderzoek (na 5 jaar). We toonden aan dat een herhaalde cytologie en hrHPV test (Hoofdstuk 7) en HPV genotypering (Hoofdstuk 5-8) eveneens gebruikt kunnen worden voor de risico stratificatie van deze vrouwen met normale cytologie. Ter illustratie van het verschil in risico op ≥CIN2 voor verschillende hrHPV typen: vrouwen met normale cytologie en HPV16/18 hadden een cumulatief 18-maands risico op ≥CIN2 van 25% terwijl het risico voor vrouwen die met een ander hrHPV type geïnfecteerd waren 5.3% was (Hoofdstuk 7).

Gebaseerd op de gegevens zoals in de voorafgaande hoofdstukken beschreven, wordt thans een kosten-effectiviteits analyse uitgevoerd
om het optimale algoritme voor het bevolkingsonderzoek op baarmoederhalskanker te bepalen waarbij op hrHPV getest wordt. Bij deze analyse worden de volgende variabelen betrokken: het bevolkingsonderzoek interval, de begin leeftijd, en de wijze waarop cytologie en HPV genotypering gebruikt kunnen worden voor een effectieve follow-up van hrHPV positieve vrouwen. Een voorbeeld van een mogelijk algoritme wordt in Figuur 1 getoond.

Figuur 1: Voorstel voor een follow-up schema voor hrHPV-positieve vrouwen die deelnemen aan het bevolkingsonderzoek op baarmoederhalskanker door primair op hrHPV te testen, waarbij gebruik gemaakt wordt van cytologie en hrHPV genotypering als triage testen.

Toekomstige ontwikkelingen: HPV vaccinatie

Ook HPV vaccinatie zou van betekenis kunnen zijn voor de preventie van baarmoederhalskanker. Twee HPV vaccins, Cervarix® en Gardasil®, die zijn gericht tegen de hrHPV typen 16 en 18, zijn commercieel verkrijgbaar in Nederland. Deze vaccins induceren hoge titers van neutraliserende antilichamen, waardoor infectie van het epitheel van de baarmoederhals met de in het vaccin aanwezige HPV typen voorkomen wordt. De vaccins zijn zeer effectief in de bescherming voor ≥CIN2 laesies veroorzaakt door HPV16/18 in vrouwen die op het tijdstip van vaccinatie negatief waren voor HPV16/18 DNA (29;30). Daarom is vaccinatie voor de sexarche (9-14 jaar) het meest optimaal. Profylactische vaccinatie met deze HPV16/18 vaccins heeft de potentie te beschermen tegen ~75% van de gevallen met baarmoederhalskanker, omdat 70% van baarmoederhalskankers aan HPV16 of HPV18 wordt toegeschreven, en een extra 5% bescherming het gevolg is van kruis-bescherming tegen HPV31 en HPV45 (29;30). De vaccins hebben geen therapeutisch effect op pre-existentie HPV16/18 infecties, noch op de door deze HPV typen veroorzaakte pre-existente CIN lesies.

Vanuit het oogpunt van volksgezondheid en met het oogmerk een optimaal beschermende effect van vaccinatie te verkrijgen, dient een hoge dekkingsgraad onder vrouwen voor de sexarche behaald te worden. Dit doel kan bereikt worden als HPV vaccinatie wordt opgenomen in het Rijksvaccinatie programma, aangeboden aan vrouwen voor de puberteit. De vraag kan gesteld worden of er nog een plaats is voor het bevolkingsonderzoek op baarmoederhalskanker in het tijdperk van profylactische vaccinatie. Aangezien profylactische vaccinatie van vrouwen voor de puberteit slechts een maximale bescherming geeft van 75% en veel van de huidige generatie volwassen vrouwen niet gevaccineerd zijn, zal het bevolkingsonderzoek baarmoederhalskanker noodzakelijk blijven. Voor de nabije toekomst mag verwacht worden dat bij voorkeur een primaire test op hrHPV de plaats van cytologie zal innemen bij het bevolkingsonderzoek baarmoederhalskanker (31)

Conclusie

In dit proefschrift presenteren we het definitieve bewijs dat primair testen met hrHPV bij het bevolkingsonderzoek baarmoederhalskanker klinisch relevant ≥CIN2 laesies eerder detecteert
De vroegere detectie van klinisch relevante CIN laesies en het 64% gereduceerde 5 jaars risico op ≥CIN2 lesies na een negatieve hrHPV test t.o.v. een negatieve cytologie test, houdt in dat het huidige bevolkingsonderzoek interval verlengd kan worden. Dit is van voordeel voor vrouwen vanwege de reductie in het aantal bevolkingsonderzoekskronden en in het aantal verwijzingen. Dit kan leiden tot een verhoogde deelname aan het bevolkingsonderzoek programma. Aangezien de vroegere detectie van ≥CIN2 zal leiden tot een reductie in persoonsjaren at risk waaronder ≥CIN2 laesies tot baarmoederhalskanker kunnen leiden, kan de eerdere detectie van ≥CIN2 ook een reductie in de incidentie van baarmoederhalskanker tot gevolg hebben (14;15). Onder de aanname dat alle partijen betrokken bij het bevolkingsonderzoek, en hieronder met name de huisartsen, voorzien worden van adequate informatie over hrHPV, wordt een bevolkingsonderzoek baarmoederhalskanker waarbij primair op hrHPV getest wordt, goed geaccepteerd en leidt het niet tot een afname in de deelname (Hoofdstuk 4). De te gebruiken hrHPV testen behoren een optimale balans te laten zien tussen sensitiviteit en specificiteit voor de detectie van ≥CIN2 en test eigenschappen behoren opgenomen te worden in de richtlijnen voor het bevolkingsonderzoek. Zulke testvereisten zijn recent geformuleerd door Hesselink (25). Thans zijn twee testen, i.e., GP5+/6+ PCR en HC2 klinisch gevalideerd en deze kunnen gebruikt worden als primaire test in het bevolkingsonderzoek baarmoederhalskanker. Gebaseerd op onze data wordt thans een volledige kosten-effectiviteits analyse uitgevoerd om te bepalen welk algoritme bij testen op hrHPV dient te worden. Bovendien lijken cytologie en HPV genotyping veelbelovende testen ter verdere stratificatie van hrHPV-positieve vrouwen en worden er model-studies uitgevoerd teneinde het meest optimale, eenvoudige en vrouw-vriendelijke follow-up algoritme te bepalen. Verwacht mag worden dat in de toekomst een moleculaire test de triage door reflex cytologie zal vervangen, waarmee de plaats van een “subjectieve techniek” definitief door een “objectieve en hoog reproduceerbare test” ingenomen zal worden.
Referenties


Dankwoord
Aan het eind van een proefschrifttraject is het tijd om achterom te kijken, en te denken aan alle mensen die bij de totstandkoming van dit proefschrift betrokken waren en die het leven aangenaam hebben gemaakt. Dit proefschrift is vooral tot stand gekomen door teamwerk.

Professor Chris Meijer, beste Chris, bedankt dat je me deze kans geboden hebt, je enorme gedrevenheid en enthousiasme zijn vaak een genoegen om mee te mogen werken en soms een kunst om mee om te gaan. Mede daardoor is het gelukt om zo’n megaproject als POBASCAM/HPVBOB op de rails te houden en tot een goed einde te brengen.

Beste Rence Rozendaal, het was een plezier om je te leren kennen en om altijd bij je terecht te kunnen. Vooral in de beginjaren van mijn proefschrifttijd, maar ook later, was je er altijd om me weg wijs te maken met de ontelbare contacten die je binnen en buiten het VUmc had opgebouwd in het voortraject, en die zeer belangrijk waren voor het goed lopen van het project. Je kunst om steeds weer dingen om te bouwen in ‘foxpro’ naar gelang de zoveelste ‘modernisering’ is ongekend. Onze discussies over ‘hpv’ waren altijd enerverend, soms verwarrend, doch altijd leerzaam, zeer zeker ook bij onze samenwerking aan mijn eerste ‘stukken’. In zijn totaliteit ben je van onschatbare waarde voor ons project.

Best Hans Berkhof, ik heb een enorme bewondering voor jouw manier om kennis te vergaren, inzicht te ontwikkelen, en deze toe te passen in de praktijk, echt ongekend. Het was ontzettend leerzaam om iedere keer weer deze inzichten te mogen horen en te bediscussiëren. Vaak voorafgegaan door dagelijkse levenszaken die in de weg zaten en waarover je op een eigen manier kon spuien alvorens over te gaan tot de berg wetenschapswerk die verricht moest worden. Aan onze samenwerking voor de ‘typeringsstukken’ en het ‘Lancet stuk’ heb ik goede herinneringen overgehouden, ook al was het niet altijd gemakkelijk.

Beste Professor Peter Snijders, je altijd goede humeur maakt het prettig om met jou samen te werken. Wanneer er weer iets moest gebeuren met typering of andere hpv-test-zaken was je er altijd om het na overleg met de analisten te regelen. Ook op ‘artikel-redigeer’ gebied kon ik altijd bij je terecht. Ik wist dat je er was op de momenten dat het nodig was, veel dank daarvoor.

Beste Saskia, ik kan me niet voorstellen hoe deze jaren waren geweest zonder jou. Samen jarenlang op een kamer, vaak in de zelfde leuke en minder leuke fasen van ons leven, die soms eng parallel liepen. Je bent een uniek persoon en ik ben blij je te hebben mogen leren kennen. Je bent vaak een enorme steun geweest in wetenschappelijke zaken, opbeurende zaken, kijk op het leven en op mensen, maar bovenal was het iedere dag weer onverwacht en verrassend met jou als kamergenoot, wat enorm heeft bijgedragen in het plezier op de werkvloer.
Beste Folkert van Kemenade, ik vond het erg prettig om met jou te hebben mogen samenwerken. Je bent een op en top diplomaat naar de buitenwereld, en dat is nodig in zo’n groot project waarin we samenwerken met allerlei partijen. Daarvoor was je van onschattbare waarde. Verder was je altijd eerlijk met je oordeel, iets waar ik ook echt wat aan heb gehad.

Beste Feja Voorhorst, dank voor de samenwerking, voor de inleiding in de statistiek, en voor de stimulatie om daarin een eigen weg te vinden.

Beste Joan, dank voor je eeuwige opgewektheid, je interesse in hoe het nou echt ging, en voor je inzet om allerlei zaken te regelen met de 240 huisartsen die aan ons project meewerken.

Beste Professor René Verheijen, dank voor medewerking om in gynaecologenland de juiste medewerking te krijgen voor ons project en voor je kritische blik op de manuscripten.

Beste collega’s van de cytologie, Aletta, Aty, Wil, Marga, Kelly, Kirsten, Monique, Fahrat, Marieke en Anneke, Daniëlle en Branko in eerdere tijden: het was heel erg fijn om al deze jaren bij jullie te mogen logeren. Er was altijd gezelligheid, en een luisterend oor in de buurt, waarvoor ik jullie enorm dankbaar ben. Branko bedankt voor de regelzaken in de begintijd en je altijd nuchtere kijk op zaken. Aletta, het was erg gezellig om je als kamergenoot te hebben en om levenszaken te overpeinzen en samen met een verwonderende blik naar de gebeurtenissen om ons heen te kijken. Aty bedankt voor je eeuwige inzet voor de hpvbob en je zorgzaamheid. Wil, Marga en Kelly, bedankt voor jullie gezelligheid, vrolijkheid en luisterend oor, het was een plezier om met jullie te werken.

Beste collega’s van de moleculaire pathologie, Nathalie, Muriël, Marjolein, Debby, René, Rick, Hans, Fatih, en in eerdere dagen, Jolein, Stephan†, Anthonie, Joyce, en Pien, het was erg prettig om samen te werken met een team zoals jullie. Er werd altijd gewerkt in goed overleg, zowel bij de dagelijkse gang van uitslagen, als wanneer er weer eens met spoed een hele serie getest moest worden. Het is enorm leuk om met een groep mensen te werken die begrip voor zaken hebben, nuchter en gezellig zijn, en bovenal meedenken. Heel erg bedankt daarvoor.

Beste Marcia, Carla en Margriet, en ook Anja en Joyce, bedankt voor al jullie werkzaamheden voor de hpvbobadministratie. Marcia je bent al vele jaren een topper hierin, enorm bedankt. Jeroen bedankt voor de snelle samenwerking wanneer er weer palgaproblemen waren.
Beste Mathilde Boon, Krijn van Groningen en Watze Ruitinga, dank voor jullie medewerking aan ons project. Beste Jur, Feya, Myra, Mirjam, Wim, Hanny, Marleen, Liesbeth, en in eerdere tijden Radboud, Deja en Frank, enorm bedankt voor de prettige samenwerking voor de hpvbob. Het was naast de routine vaak best een geregel maar wel plezierig om de zaken goed en snel te regelen via een telefoontje of een emailtje.

Beste Dorien en Murat: zet’m op om jullie projecten met een succesvol proefschrift af te sluiten!, het was en is plezierig om met jullie te werken.

Beste Bart, bedankt voor de samenwerking en de gezelligheid, dat we het maar spetterend mogen vieren op ons feest.

Beste collega-pathologen-in-opleiding en pathologen, bedankt voor jullie interesse, steun en begrip voor de soms moeizame laatste loodjes. Ik heb enorme zin me nu nog meer in de klinische pathologie te gaan onderdompelen.

Beste Jaap en Ron, bedankt voor jullie vrolijkheid en interesse, en Jaap bedankt voor de voorbewerking voor het drukken van dit proefschrift waarbij je mijn redder in nood was met het oog op de drukkwaliteit.

Beste paranimfen Maaike en Marjolein: Maaike ontzettend bedankt voor je steun, tips, interesse, je 'typering-stuk-ideeën, je kritische blik op de manuscripten waar je aan mee hebt gewerkt. Ik heb geleerd van je onderzoekende manier van denken en je relativeringsvermogen. Beste Marjolein, bedankt voor je altijd oplettende manier van werken, bij analyses, bij de administratie en bij andere zaken, en voor je gezelligheid en het meedenken. Het was erg prettig om met je samen te werken.

Beste Evelyn, Lisette en Susanne, bedankt éénieder dat jullie al 18, 32, en 18 jaar echte vriendinnen zijn, voor het plezier dat we samen beleven, en de steun die ik altijd bij jullie kan vinden wanneer ik dat nodig heb. Beste Ilonka, beste schoonzus, bedankt voor je interesse, steun en gezelligheid.

Beste papa en mama, ontzettend bedankt voor jullie onvoorwaardelijke steun bij de keuzes die ik maak. Onbewust is dat een heel belangrijk gevoel wat iemand juist de mogelijkenheden geeft om zich te ontwikkelen. Verder is het van onschattbare waarde hoe jullie het gezinsleven van Edwin en mij steunen, en hoe jullie samen met ons genieten van de meisjes.

Lieve Edwin, het leven is fantastisch met jou, dank voor je relativeringsvermogen, je eeuwige steun en je liefde. De manier waarop wij elkaar stimuleren in ons werk en ons gezin samen beleven maakt dit alles mogelijk.

Lieve Emma en Charlotte, jullie zijn de meest bijzondere fascinerende meisjes die er zijn, het is heerlijk om met jullie het leven te mogen beleven.
Curriculum Vitae

Nicole Bulkmans was born on 26th August 1971 in Rucphen. In 1989 she graduated form high-school at the V.W.O. of the Katholieke Scholengemeenschap Etten-Leur e.o. in Etten-Leur and started her medical school at the Erasmus University in Rotterdam. During her study she performed scientific research at the Presbyterian Joint Hospital in Uburu in Nigeria, and at the department of Obstetrics and Gynaecology of the Academic Hospital Uppsala in Uppsala in Sweden. In 1997 she graduated from medical school. She worked as a medical trainee at the departments Internal Medicine and Intensive Care of the Sint Franciscus Gasthuis in Rotterdam, and at the department of Obstetrics and Gynaecology of the Erasmus Medisch Centrum in Rotterdam. In April 2001 she stared her research project at the department of Pathology of the VU University Medical Center in Amsterdam supervised by prof.dr. C.J.L.M. Meijer, resulting in this thesis. Since December 2006 she started her specialist trainee in Clinical Pathology at the VU University Medical Center in Amsterdam. She lives in Hilversum together with her husband Edwin Leenheer, and their two daughters Emma (2003) and Charlotte (2005).
List of Publications


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