HPV TESTING ON SELF-COLLECTED CERVICO-VAGINAL MATERIAL: A NEW WAY OF WOMAN-FRIENDLY CERVICAL SCREENING

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HPV testing on self-collected cervico-vaginal material: a new way of woman-friendly cervical screening

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List of Abbreviations

HPV	Human Papillomavirus
CIN	Cervical Intraepithelial Neoplasia
PAP	Papanicolau
SIL	Squamous Intraepithelial Lesion
HSIL	High-grade Squamous Intraepithelial Lesion
LSIL	Low-grade Squamous Intraepithelial Lesion
HC2	Hybrid Capture 2
PCR	Polymerase Chain Reaction
PALGA	Pathologisch Anatomisch Landelijk Geautomatiseerd Archief
RR	Relative risk
GP	General practitioner
LLETZ	Large Loop Excision of the Transformation Zone

Contents

Chapter 1 *Introduction*

Chapter 2 *Cytology history preceding cervical cancer diagnosis: a regional analysis of 286 cases*

Chapter 3 *HPV testing on self-collected cervico-vaginal lavage specimens is an effective screening method for non-attendees of cervical screening: results from the PROHTECT cohort study*

Chapter 4 *Experience with high-risk human papillomavirus testing on vaginal brush-based self-samples of non-attendees of the cervical screening program*

Chapter 5 *Offering self-sampling for human papillomavirus testing to non-attendees of the cervical screening programme: Characteristics of the responders*

Chapter 6 *HPV16 and increased risk of recurrence after treatment for CIN*

Chapter 7 *Discussion & Summary*

Chapter 8 *Nederlandse samenvatting*

List of publications

Acknowledgement

Curriculum Vitae

Chapter 1

Introduction

1.1 Cancer of the uterine cervix

1.1.1 Epidemiology of cervical cancer
1.1.2 Anatomy of the uterine cervix
1.1.3 Precursor lesions and cervical cancer

1.2 Human papillomavirus (HPV)

1.2.1 Causative agent of cervical cancer
1.2.2 HPV genome and taxonomy
1.2.3 Prevalence of HPV infections
1.2.4 Viral life cycle and transforming HPV infections
1.2.5 Concept of cervical carcinogenesis

1.3 New ways to prevent cervical carcinoma

1.3.1 Organised screening programme of The Netherlands
1.3.2 Primary prevention by prophylactic HPV vaccination
1.3.3 Secondary prevention by HPV DNA testing
1.3.4 Self-sampling
1.3.5 Triage of hrHPV-positive women

1.4 Aim and outline of this thesis

1.1 Cancer of the uterine cervix

1.1.1 Epidemiology of cervical cancer

Cervical cancer is the second most common female cancer in the world. Worldwide, an estimated 437,000 new cases of invasive cervical cancer are diagnosed yearly, of which more than 80% occur in developing countries.(1) More than 233,000 women die from cervical cancer each year. Thus, worldwide cervical cancer is a major cause of death, accounting for 6-10% of all cancer related deaths in women.(2) The age-standardized incidence rates in developed countries are less than 14 per 100,000, compared to up to 43 per 100,000 in developing countries. This difference in incidence may partly be explained by introduction of cervical cancer screening programmes in the 60s and 70s of the previous century in developed countries.(3-5)

The Netherlands is among the countries with one of the lowest incidence of (7.9 new cases per 100,000 women in the year 2007) and almost the lowest mortality from cancer of the uterine cervix (1.7 per 100,000 women).(6) Yearly, approximately 630 cases of cervical cancer are diagnosed, while the yearly number of death is about 225 deaths.(7) The risk for a Dutch woman to be diagnosed with or die from cervical cancer before she reaches the age of 75 is 0.46% and 0.13%, respectively.(8) From 1988 (11.8 per 100,000 women yearly) to 2000 (8.2 per 100,000 women years) an incidence reduction of 2.7% was observed, mainly caused by a decrease in the incidence of squamous cell carcinoma (SCC).(9) Besides a natural decline due to better sexual hygiene after the second world war, the decrease of SCC could for a large part be explained by introduction of organized cervical screening.(10)

Squamous cell carcinoma (SCC) is the most common histotype of cervical cancer (80%-85%%). The second most common type is cervical adenocarcinoma (AdCa), which constitutes approximately 10%-15% of cervical cancers. The remaining 5% are rare histotypes, including small cell carcinoma.(11)

The prognosis depends on the patient's age and general health, the stage and type of the cervical cancer.

1.1.2 Anatomy of the uterine cervix

The cervix is the entrance of the uterus and is divided into the vaginal portio (ectocervix) and the endocervix. The ectocervix and the vagina are covered by a stratified nonkeratinizing squamous epithelium continuous with the vaginal vault. The endocervical canal is lined by tall *columnar* cells. Somewhere on the cervix the two cell types, squamous cells and columnar cells, meet at a place microscopically called the *squamo-columnar junction* (SCJ). The position of the junction is variable because of both the cervical anatomy and age-related hormonal influences. From puberty onwards sqamous metaplasia replaces the columnar epithelium at the SCJ. The area between the original SCJ and the current SCJ is called *transformation zone (TZ).* The TZ is thought to be most susceptible to high-risk human papillomavirus infection and subsequent

(HPV)-mediated transformation of cervical cells. This is typically the area in which abnormal growth or dysplasia develops.

1.1.3 Precursor lesions and cervical cancer

Cervical SCC typically develops in the mucosa of the TZ via premalignant precursor lesions, so called *cervical intraepithelial neoplasia* (CIN). These histologically recognizable foci of abnormal growth or dysplasia can be classified on the basis of progressive atypia of the epithelial cell lining. The classification system has 3 levels; CIN 1 (mild dysplasia), CIN2 (moderate dysplasia), and CIN3 (severe dysplasia). CIN1 means that the lower 1/3 of the total thickness of the squamous epithelium shows atypia (mild dysplasia). For CIN2 the lower 2/3 of the total thickness of the epithelium (moderate dysplasia), and for CIN3 the complete epithelial layer shows atypia (severe dysplasia and carcinoma in situ).

This three-tiered classification has recently been simplified to a two-tiered classification on the basis of a two-tiered decision management (observation vs. surgical treatment). The CIN I has been renamed into *low-grade squamous intraepithelial lesion* (LSIL), and CIN II/III into *high-grade squamous intraepithelial lesion* (HSIL). LSILs have a low progression rate to invasive carcinoma, and in clinical practice are therefore not treated, . Instead surveillance takes place of women with LSIL. HSILs are recognized as immediate precursors of cervical SCC, and therefore surgically treated,e.g. by large loop excision of the transformation zone (LLETZ) in order to prevent cervical cancer.

Cervical AdCa arises within glands located in the endocervix. The different stages of precursor lesions of cervical AdCa, i.e *cervical intraepithelial glandular neoplasias* (*CIGN*) grade 1 and 2 are histologically poorly defined and therefore their natural history not completely understood. The best defined precursor lesion is CIGN 3 or *adenocarcinoma in situ* (*ACIS*), which requires treatment. As is the case for CIN2/3 and SCC, high-risk HPV infection is necessary for development of ACIS and AdCa (see chapter 1.2).(12)

1.2 Human papillomavirus (HPV)

1.2.1 Causative agent of cervical cancer

The first suggestion that cervical cancer was a sexually transmitted disease was done by Rigoni-Stern in the 19th century.(13) However, it was not until mid-70s of the 20th century that the role of HPV in the development of cervical carcinoma was discovered by Nobel price-winner Harald zur Hausen.(14) Many studies have supported the causative role of an infection with high-risk HPV (hrHPV) in the development of high-grade premalignant cervical lesions(15), and cervical cancer(16-18) The hrHPV prevalence in cervical carcinomas is $\geq$99.7%.(19) The World

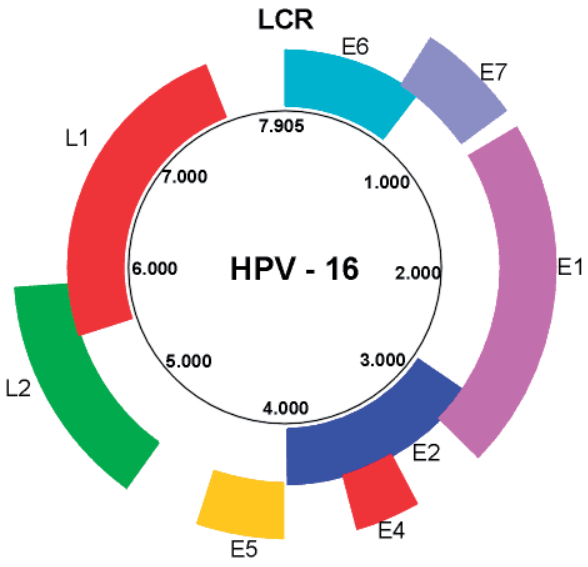
Health Organization accepted an infection with hrHPV as the primary cause of cervical cancer in 2005.(20)

1.2.2 HPV genome and taxonomy

HPVs are double-stranded DNA viruses of approximately 8,000 base pairs wrapped into a 45-55 nm sized spherical protein shell (capsid). HPVs belong to the family of Papillomaviridae,(21;22) which comprise papillomavirus (PV) types isolated from humans, non-human mammals, birds and reptiles. HPVs are strictly epitheliotrophic, and can be subdivided into cutaneous (predominantly infects the skin) and mucosal types based on their preferential site of infection. The latter types do infect the mucosa of the anogenital, respiratory and/or upper digestive tract.(23;24) This thesis will focus on the mucosal types.

The HPV DNA contains two coding regions, i.e., an early region encoding proteins necessary for viral replication (E1, E2, E4, E5, E6, E7) and a late region encoding the major and minor viral capsid proteins (L1 and L2), and one non-coding long control region, the latter of which contains regulatory elements (see Figure 1).

Figure 1: *Genomic organization of HPV*



To classify a new HPV type, subtype, or intratype variant, the homology in the genomic sequence of L1, E6 and E7 open reading frames is taken into account. Sequence variation of

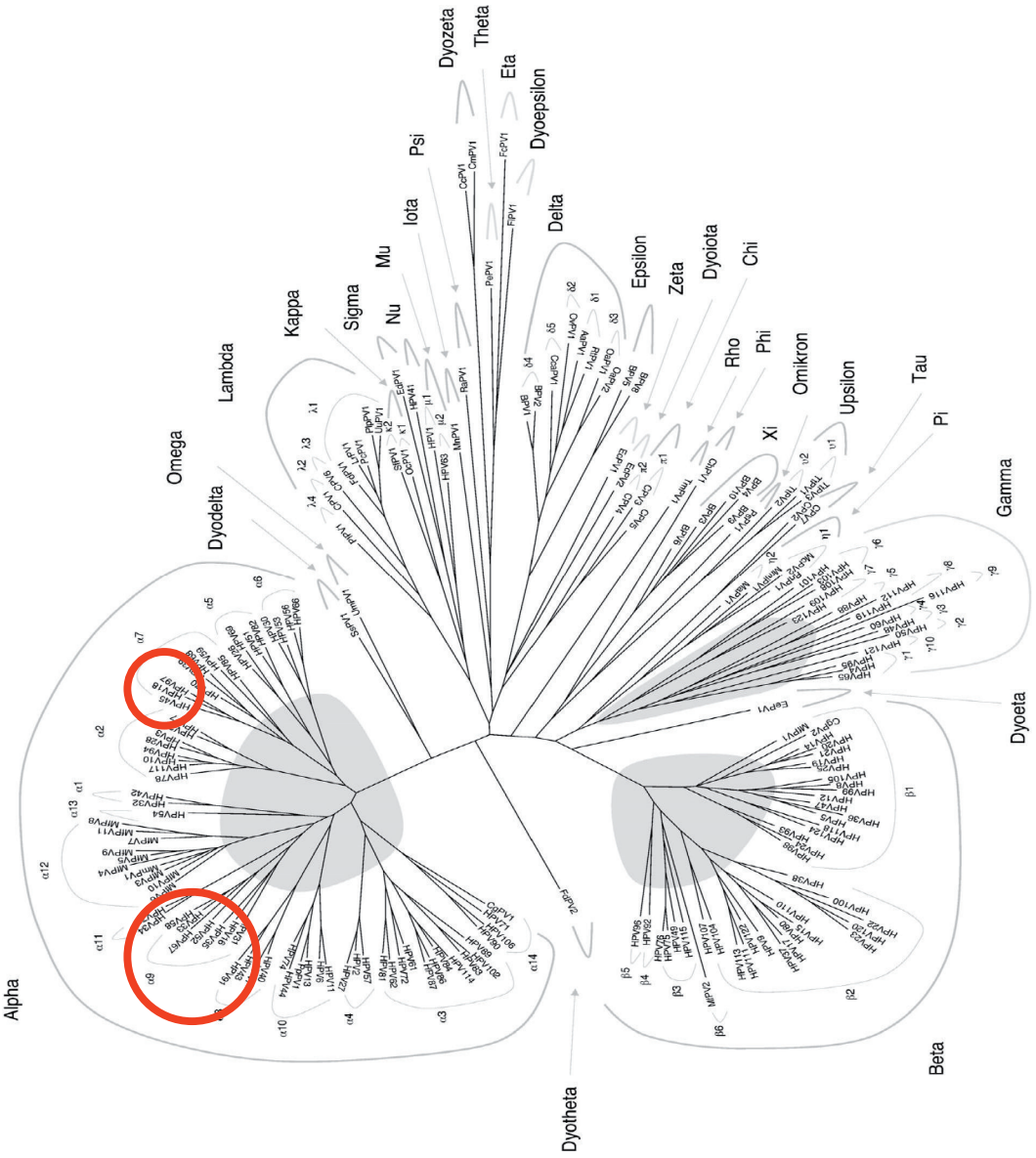
>10%, 2-10%, and <2%, classifies a new type, subtype, or intratype variant, respectively. To date, more than 120 HPV types have been identified.(22;25;26). The HPV types can be categorized into species, and these species into genera (see Figure 2).

The majority of HPV types, so-called low risk types, are associated with benign wart-like lesions. A subset of HPVs may eventually cause malignant transformation of the infected epithelium, and these types are therefore designated as high-risk (hr), or oncogenic types. The International Agency for Research on Cancer (IARC) has categorized HPV types according to their carcinogenicity to humans into different classes(27). The oncogenic potential differs between hrHPV types. The types found most frequently in cervical cancer (HPV-16, 18, 31, 33, 35, 45, 52, 58) and four types less constantly found (HPV-39, 51, 56, 59) are categorized into in Group 1 (Table 1). Among these, HPV16, and -18 confer the highest risk of high-grade CIN lesions and cervical cancer (≥CIN2).(28-31) HPV-68 was classified as "probably carcinogenic to humans" (Group 2A) with limited evidence in humans but strong mechanistic evidence. The remaining types of HPV in the high-risk alpha species were classified as "possibly carcinogenic" (Group 2B). Finally, HPV-6 and HPV-11, which belong to the alpha-10 species, were not classifiable as carcinogenic for humans (Group 3) on the basis of inadequate epidemiological evidence and absence of carcinogenic potential in mechanistic studies. Nonetheless, some studies suggest HPV6 and -11 as possibly carcinogenic to human beings, which warrants further investigation.(32)

1.2.3 Prevalence of HPV infections

Genital HPV infections are very common. The HPV prevalence depends on geographic area and age. The HPV prevalence is highest among women between the age of 16 and 25 years (approximately 20%). The highest prevalence rates have been detected in Africa and South America, while the prevalence rates are the lowest in Europe and Asia.(33) Worldwide, the HPV prevalence in women with normal cytology at any moment in time is approximately 10%. This indicates that HPV is one of the most common sexually transmitted infections.(34) It is estimated that about 80% of sexually active individuals encounter an HPV infection during their life, most of which pass unnoticed.(35) Moreover, 80% of all hrHPV infections will not result in lesions and are referred to as transient infections. The remaining 20% results in morphological changes, read as CIN1, CIN2 or CIN3 lesions by pathologists. Often these lesions will regress following clearance of the virus, but is highest in CIN1 and lowest in CIN3 lesions. Thus, genital HPV infections are very common, but only a few HPV-infected individuals (~1-3%) ultimately show progression to invasive cancer.(36)

Figure 2: *Phylogenetic tree of Papilloma viruses*



Adapted from Bernard et al, Virology 2010

Table 1: Classification of HPV types according to oncogenic potential

Group	Alpha HPV types α7, α 9, α10	Comments
1	**Alpha 9:** 16	*Most potent HPV type, known to cause cancer at several sites*
1	**Alpha 5:** 51 **Alpha 6:** 56 **Alpha 7:** 18, 39, 45, 59 **Alpha 9:** 31, 33, 35, 52, 58	*Sufficient evidence for cervical cancer*
2A	**Alpha 7:** 68	*Limited evidence in humans but strong mechanistic evidence for cervical cancer*
2B	**Alpha 5:** 26, 82 **Alpha 6:** 53, 66 **Alpha 7:** 70, **Alpha 9:** 67	*Limited evidence in humans for cervical cancer*
2B	**Alpha 7:** 85, 97	*Classified by phylogenetic analogy to HPV types with sufficient or limited evidence in humans*
3	**Alpha 10:** 6, 11	*Most commonly associated with benign lesions such as genital warts and mild dysplasia of the cervix*

1.2.4 Viral life cycle and transforming HPV infections

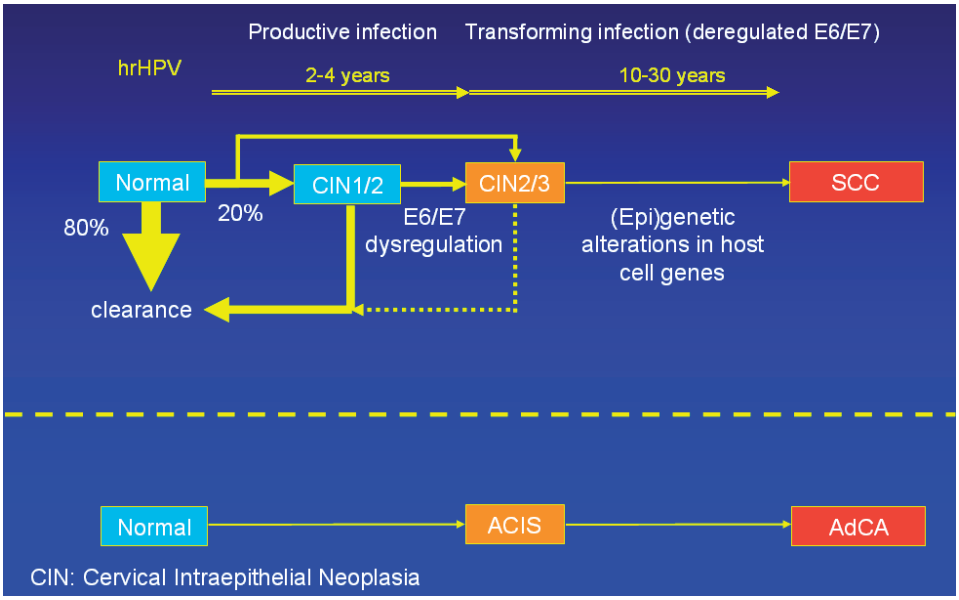
HPV infections normally have a tight connection to the differentiation process of the host cell. The viral genome is maintained as an episome in the basal cells, viral DNA replication occurs in the suprabasal layers, encapsidation takes place in the upper layers of the host epithelium, and finally viruses are shed when the superficial epithelial cells die. Besides the viral proteins E1 and E2, which are essential for viral replication, the virus relies entirely on the host cell DNA replication machinery for viral DNA synthesis. Viral E5, E6 and E7 proteins are needed to create conditions that allow viral replication in differentiated non-dividing epithelial host cells, in which the DNA replication machinery is normally not activated. This form of HPV infection in

which new viral particles are formed is referred to as a *productive infection*. Such infections may give rise to mild or moderate histomorphological abnormalities (CIN1 and CIN2), but usually do not result in development of ≥CIN3.

On the other hand, *transforming infections* are associated with high-grade CIN lesions and cervical cancer. These infections are characterized by excess expression of the viral E6 and E7 oncogenes in the proliferating cell compartment of the epithelium and abortion of the normal viral life cycle. Interactions of the viral oncoproteins with cellular tumor suppressor proteins, particularly E6 with p53 and E7 with pRb in these proliferating cells provide a basis for genomic instability. The accumulation of (epi)genetic alterations trigger malignant transformation (see Figure 3).(37;38)

It is thought that transforming infections develop through progression of a minority of productive HPV infections by a mechanism not fully understood. Genomic loss, integration of the virus in the cellular genome and Methylation of E2 binding sites are thought to play a role in progression to a transforming infection.

Figure 3: *progression model of cervical carcinoma*



1.2.5 Concept of cervical carcinogenesis

As a result of a transforming hrHPV infection, CIN3 lesions may develop relatively fast, i.e. within 3 years.(39;40) Up to 50% of CIN3 lesions can progress to cancer if left untreated, a

process which may take 10-30 years.(3;41) Thus, although hrHPV infections are necessary to cause cervical cancer, it is far from sufficient. Additional genetic aberrations, including the accumulation of (epi)genetic alterations, is required for a precursor lesion to become invasive.

1.3 Ways to prevent cervical carcinoma

1.3.1 Organised cervical screening programme

The fact that cervical cancer develops via premalignant precursor lesions which can be treated effectively, offers possibilities for screening to prevent cervical cancer development. In The Netherlands, cervical cancer screening using the Pap smear was introduced in the beginning of 1970's. The target was women between the age of 35-55 years with a smear taken every 3 years.

It was clear that this programme failed to meet standards set by the WHO. The system became nationwide only in 1988 and the programme mainly reached younger women, as the age target was set on 35-55 years of age. Furthermore, in 1990 the invitational reach of organised cervical screening programme was approximately 85%.(7) Approximately 69% of invited women did participate the screening programme. In addition, many cytology tests were read as abnormal (~14%), resulting in many repeat tests. Also the timely compliance rate with recommendations for a repeat smear was less than 50%.

In order to amend the lack of full coverage, the underscreening of invited higher age groups, the overscreening of lower age groups and to limit the number of repeat tests, the secretary of health council ordered a revision of the current programme in 1991. However, the screening programme still showed too much lack and still did not meet standards set by WHO.

In 1996, the Dutch cervical screening organisation was revisited again. To avoid overscreening and to allow extension of the programme to older age stata all women aged between 30 and 60 years (instead of 35-55 years) were invited every 5 years. Thus, the number of invitations per life time did not change.(42)

As a result, the 5-year coverage in the age range 30-64 years increased from 69% in 1994 to 77% in 2003. The percentage of smears resulting in a recommendation for a repeat smear decreased from 10 to 2. The percentage of timely compliance with recommendations for a repeat smear increased from 47 to 86, while that of smears with an immediate referral recommendation remained the same. Also, the percentage of women receiving an invitation for screening rose from 85% to 98%.(7) Another important change in the restructuring was implementation of a new follow-up algorithm combined with a more consistent classification of the cytological smear results (CISOE-A classification). Briefly, the CISOE-A classification interprets smears using a rating system including information on specimen composition, inflammatory characteristics, and adequacy of the smear. The letters C (composition), I (inflammation), S (squamous), O (other and endometrium), and E (endocervical glandular epithelium) are used to indicate the composition and morphology of the smears. As a result the

percentage of women with borderline or mild dyskaryosis (BMD) decreased from 11.3% in 1990 to 2.6% in 2000 ($p < 0.001$)(43). The CISOE-A interpretations can be easily "translated" into the Bethesda classification (see Table 2; adapted from Bulk et al, 2004(43)).

Moreover, in case of BMD, women received two additional smears at 6 and 18 months: if any of these repeat smears was read as outside normal limits (BMD or worse) women were to be referred for colposcopy directed biopsy (so called *indirect* referral). Women with cytology results worse than mild dyskaryosis were referred immediately to a gynaecologist (so called *direct referral* based on one smear result). Finally, reimbursement for general practitioners (GPs) was, according to the algorithm, limited to programme smears only, giving a strong disincentive to spontaneous or opportunistic smears. The last major change in the organised screening programme was recently introduced when the number of founded screening organisations was reduced from 12 to 5, and these were given full governance in order to save costs and maintain quality.

Table 2: CISOE-A classification

S	O	E	Pap	Description	Bethesda 2001	
0	0	0	0	Inadequate	Unsatisfactory for evaluation	
1	1	1–2	1	Normal	Negative for intraepithelial lesion or malignancy	
1	2	1–2	1	Normal	Atrophy, negative for intraepithelial lesion or malignancy	
2–3	3	3	2	Borderline dyskaryosis	ASC-US/ASC-H	AGC
4	4	4	3a1	Mild dyskaryosis	ASC-H/LSIL	AGC favour neoplastic
5	5	5	3a2	Moderate dyskaryosis	HSIL	AGC favour neoplastic
6	6	6	3b	Severe dyskaryosis	HSIL	AGC favour neoplastic
7	–	7	4	Carcinoma in situ	HSIL	AIS
8–9	7–8	9	5	Carcinoma	Squamous cell carcinoma	Adenocarcinoma

AGC, atypical glandular cells; AIS, endocervical adenocarcinoma in situ; ASC-H, atypical squamous cells cannot exclude HSIL; ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HSIL, high grade squamous intraepithelial lesion, encompassing CIN2–3; LSIL, low grade squamous intraepithelial lesion, encompassing CIN1.

Despite the improved changes, the effects of screening programmes on carcinoma incidence (and mortality) are still being debated. Incidence of and mortality from SCC started to decrease already in the fifties before the organised screening programme was introduced.(10) The decrease may partly be caused by natural declining, probably caused by improved sexual hygiene. Moreover it is difficult to prove (without randomised controlled trials) that the introduction of screening has contributed to the decrease in the incidence SCC in the Netherlands.(9) In addition, modelling studies have suggested that the Dutch screening programme would reduce the risk of dying from cervical cancer by 75%, under the assumption that all women would adhere to the programme and comply with all 7 invitations. Yet, this aim seems increasingly unlikely.(44)

However a recent British publication described that without cervical screening an epidemic of cervical cancer would have occurred in the UK.(3)

As mentioned above, the incidence of cervical cancer in The Netherlands decreased between the year 1988 and 2000.(9) However, the incidence of cervical cancer cases does not seem to have reached its bottom.(45) The main cause is the limited compliance of women to participate, reflected by either complying with screening at less than the recommended frequency or non-participation at all (non-responders; see below). Yet, other causes cannot be excluded, such as an increase in carcinoma incidence owing to a changed lifestyle.(3;45) In addition, failures in the process (smear taking, smear handling, screening and/or interpretation of abnormal cells, reporting failures), cause failures in diagnosis ,treatment and follow-up monitoring.(46)

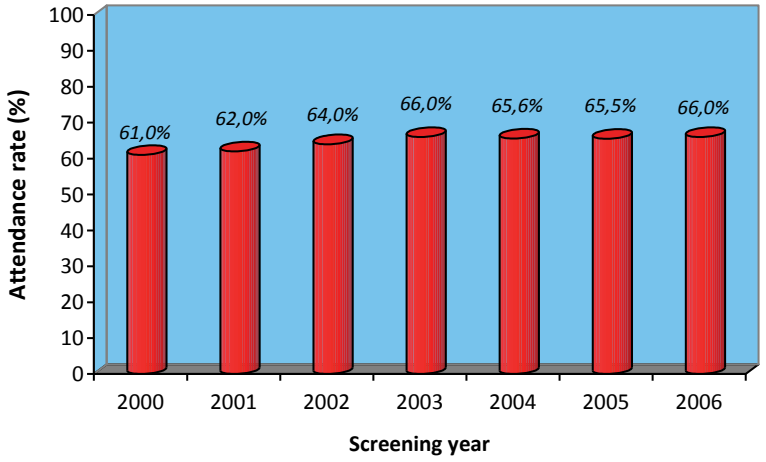
An additional complication is the presumed shift in histotype from SCC to AdCa of the uterine cervix. Although the original modelling was done for SCC, the incidence of AdCa either remained the same or even increased in some countries.(47-49) For patients with AdCa the relative risk (RR) of death is 1.6 times higher compared to patients with SCC (95% CI 1.2 – 2.1).(9) Cytology seems to be more efficient in detecting (pre-)malignant stages of SCC than those of AdCa.(9)

Every year, approximately 800,000 are invited, but ~528,000 women (~66%) do attend to the organised screening programme in the Netherlands (see Figure 5. Source: RIVM; www.rivm.nl/bevolkingsonderzoeknaarbaarmoederhalskanker/onderwerpen).

A considerable number of women are not screened at all.(50) In addition, approximately 65% of women participates the screening programme (see Figure 4), 12% of women have opportunistic screening or are screened by indication. Thus, nearly 23 % of the invited women do not undergo any screening. Several studies have portrayed the screening histories of women with histologically confirmed cervical cancer.(51-53) The conclusion of these studies was that lack of an adequate cervical screening history (i.e. not being screened) was the major risk factor, but also misreading of smears, inconsistent or delayed follow-up after an abnormal smear or a not representative smear were important reasons for failure to detect (precursor lesions of) cervical cancer.

Previous study showed that in the period 2000-2003, the number of cervical cancer was ~650 per year in The Netherlands. The mortality from cervical cancer was ~230 women per year.(7) More than half of these women (~55%) was not screened in the previous round prior to the histologically confirmed diagnosis. Possible reasons for the non-participating may be the inconvenience of having a smear taken, the time required, the estimated risk a woman considers herself to have and the discomfort.(54) Increasing screening participation, therefore, seems essential to decrease the number of cervical cancer even more.(55;56)

Figure 4: *attendance rate of the organised screening programme*



Source : RIVM

1.3.2 Primary prevention by prophylactic HPV vaccination

On the basis of the insight that cervical cancer is caused by hrHPV types, two new directions have emerged for the prevention of cervical cancer: primary prevention by prophylactic vaccination against high-risk types HPV-16 and HPV-18, which are worldwide responsible for approximately 70% of cervical cancers(57) and secondary prevention by cervical screening with HPV DNA testing.

The currently available prophylactic HPV vaccines are based on virus like particles (VLPs) of HPV16 and HPV18 composed of HPV L1 proteins VLP's develop spontaneously by folding of the isolated HPV 16 and 18 L1 proteins. The currently available vaccines are the quadrivalent vaccine Gardasil® (MSD), which besides HPV16 and HPV18 also contains VLP's of non-oncogenic types HPV6 and HPV11 and the bivalent vaccine Cervarix®; (GSK) vaccines. A VLP is geometrically and antigenically almost identical to the native virion. Thus, VLPs resemble the actual virus morphologically but cannot induce infection as these do not contain viral DNA. Once introduced intramuscularly, VLP vaccines generate high levels of systemic anti-HPV L1 immunoglobulin G (IgG) antibodies. There is now substantial evidence that the serum-neutralising IgG antibodies induced by these HPV-VLP vaccines and produced by plasmacells in bone marrow and lymph nodes reach the anogenital epithelial surface through diffusion. They

prevent attachment of the HPV particle to the epithelium and as such an HPV infection. They provide protection against persistent infection of epithelial cells with HPV types represented in the vaccine and against incident and persistent CIN2+ lesions caused by the vaccine HPV type. Protection is in principle type-specific, but cross-reactivity against HPV45, partially against HPV31 and HPV33 has been proven(58) and may occur against other phylogenetically related HPV types because they share cross-neutralisation epitopes.(59) Neither one of the vaccines is effective in persons that are already infected with HPV, and consequently these vaccines have no therapeutic effect. In public prevention programmes, HPV vaccination should therefore be confined to preadolescent girls and/or boys that are not yet sexually active and considered immunologically naive for HPV. Modelling has shown that vaccination of girls is probably sufficient to attain sufficient immunity in a population (herd immunity).

Indeed, many countries have started vaccination programmes, primarily targeting preadolescent girls.(60) In the Netherlands, the HPV vaccine has been incorporated into the national vaccination programme and started with vaccination of girls aged 12 years in 2010 (*http://www.rivm.nl*).

1.3.2 Secondary prevention by HPV DNA testing

The effect of implementing HPV testing has been investigated in several population-based screening trials, the results of which indicate that HPV DNA testing detects 50% more high-grade lesions as compared to cytology.(61) HPV DNA testing detects clinically relevant lesions at an earlier stage. The result is that women with a negative HPV tests result have a markedly decreased risk of developing high-grade CIN lesions(62-74) and cervical cancer(75) in the next screening round (three or five years later, compared to women with a cytologically negative smear, permitting less frequent screening.

The HPV test used in these trials are the hybrid capture 2 (HC2) test and the GP5+-6+-PCR-EIA assay, which are considered clinically validated for screening purposes.(76;77) The *hrHPV HC2* is a signal amplification method in a liquid-phase format and uses a mixture of full-length RNA probes representing 13 HPV types (i.e. HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) to hybridize to HPV DNA in heat-alkaline-denatured samples. RNA/DNA hybrids are detected by peroxidase-labeled antibodies and visualized by electro-chemiluminescence (ECL).(78) The *GP5+/6+PCR-EIA* test is a PCR-based assay, amplifying L1 DNA of a broad spectrum of HPV types. The read-out system involves an enzyme immunoassay (EIA) staining procedure using oligoprobe cocktails representing high-risk and / or low-risk HPV types.(79) Presently a commercial version is available.(80)

Finally, many more different HPV tests are available. However, it was already recognised in 2003 that it is of utmost importance to define criteria that should be fulfilled by an HPV test before the implementation of such a test in a population-based cervical cancer screening

programmes(81) and such guidelines recently have been made by an international consortium.(76)

1.3.4 Self-sampling

As mentioned in paragraph 1.3.1, the compliance rate of women attending regular screening programme needs to be increased, given a substantial non-attendance rate of ~23% and the fact that more than half of the diagnosed cervical carcinomas is found in this group. Several methods may lead to improvement of participation by invited women in organised screening programmes. Hermens et al. have shown that women are more willing to attend when invitations are sent by their general practitioner instead of the municipality.(82) Another alternative to influence the effectiveness of the screening programme is computerization and, to a lesser degree, more intensive support to practices and delegation of many clinical tasks to the practice assistant.(83)

Most importantly, simplifying the procedure for women by offering a self-sampling device for collecting cervico-vaginal material may positively affect the participation rate.(54;84-87)This method potentially can lower the threshold for participation that normally is not reached because of embarrassment, language difficulties, fear or lack of time during daytime for working women, and/or culture differences. Epidemiological studies in which participants were asked for collection preference have shown that women prefer self- collection over physician-collection.(88-92) Time and place of sampling, privacy and ease of sampling have been mentioned as advantages of self-sampling. Even 40% of older women not participating in organised screening preferred performing self-sampling.(93) This could lead to a reduction of cervical cancer incidence by about 16% among postmenopausal women, if self-sampling would be offered.(94) Thus, there is a basis for HPV testing on self-sampled of vaginal- or cervico-vaginal material in cervical cancer screening.

Self-collected vaginal samples are not suited for accurate cytological assessment, because insufficient intact cervical cells are represented in these specimens and cytological preparations may be of poor quality.(95), resulting in too low sensitivity for cervical lesionsMoreover, liquid-based cytology (LBC) preparations of self-sampled specimens showed a poor concordance with cytology on conventional cervical smears taken by a physician and revealed much lower sensitivities for high-grade cervical disease.(96) Thus, cytology is not an option for self-sampled cervico-vaginal specimens.

Conversely, sufficient evidence has been collected that self-sampling is as sensitive as physician-obtained sampling to detect hrHPV.(90;96-99) Moreover, hrHPV testing on self-sampled material has been demonstrated to be at least as sensitive for detection of $\geq$CIN2 as cytology on cervical sampled material with endocervical brush obtained by the physician.(54;96;100-102) In fact, highly concordant results can even be obtained between

hrHPV testing on self-sampled cervico-vaginal material and physician-sampled specimens at the level of ≥CIN2.(91;103-105)
All these data open possibilities to offer self-sampling for hrHPV testing to non-attendees of the cervical screening program as a method to increase compliance. Indeed, in a pilot study we found that offering self-sampling for hrHPV DNA testing to the non-attendees in the cervical screening programme is a feasible method.(85) This study showed that ~30% of the non-attendees did respond by submitting a self-collected sample. This pilot study therefore indicates feasibility of offering hrHPV self-sampling to non-attendees, which warrant further investigations on effectiveness.

1.3.5 Triage of hrHPV positive women

The consequence of primary hrHPV testing for cervical screening, however, is the 4-6% lower specificity of this assay, as compared to cytology, for high-grade cervical lesions. The number of false-positive results, i.e. positive hrHPV results without presence of high-grade precursor lesions may lead to unnecessary follow-up and a considerable increase in colposcopies. In order to keep follow-up procedures and associated costs within acceptable limits additional triage steps of hrHPV positive women are necessary. Cytology, HPV genotyping, viral load analysis, p16 INK4A staining, E6/E7 expression analysis, and promoter methylation analysis of tumor suppressor genes have been proposed as triage tools.(106-108) At present cytology has been shown the best triage tool to identify the women who are in need of immediate colposcopy. However, because the 2 years-risk of ≥CIN3 of hrHPV positive women without cytological abnormalities is still above the level of acceptance (a CIN3+ risk of 42.2% (95% confidence interval (CI): 36.4-48.2), whereas the hrHPV-positive women with normal cytology had a much lower risk of 5.22% (95% CI: 3.72-7.91) with 1% for CIN3+ according to the Dutch screening guidelines a repeat cytology test after 6 or 12 months may be used to further decrease the number of colposcopy).(109) In future screening, it is likely that the role of cytology becomes more and more limited. A promising alternative to cytology is p16INK4A immunostaining.(110) Also promoter methylation analysis of tumor suppressor genes like CADM1 and MAL have yielded promising results but their value needs further confirmation in larger studies.(111;112) Such molecular markers could be of particular value when considering self-sampled cervico-vaginal samples for which cytology is not an effective option (see next paragraph). When proven successful promoter methylation markers will open the way for fully non-morphological screening.

1.4 Aim and outline of this thesis

Despite well-organized cervical screening, still approximately 628 cases of cervical cancer are diagnosed in the Netherlands each year. It is important to elucidate why screening may not be fully effective and to investigate ways to improve the cervical cancer screening programme.

We wondered what the influence of screening history was in women who acquired cervical cancer. Special attention was paid to attendancy. Therefore in **Chapter 2** we analyzed women with cervical cancer between 2005–2007 for cytology history preceding carcinoma, hierarchically arranging cytology history (if present) into three groups: 'screened (between 1 and ≤6 years prior to diagnosis)', 'work-up (<1 year prior to diagnosis)' and 'underscreened', (>6 yrs prior to diagnosis). For screen- and work-up smears we analyzed timeliness. FIGO-stage were measured in relation to cytology history. Women with cervical cancer are underscreened and have poor timeliness in case of abnormal cytology. Being un- or underscreened correlates significantly with higher cervical cancer stages, especially in older women (aged ≥49 years; $p<0.001$). We concluded that Improvement of attendancy is needed to meet the standards of quality for screening programmes.

Enhancing participation is important in achieving optimal protection from screening programmes. We therefore assessed the effect of offering self-sampling for hrHPV testing to non-responders of the regular cervical screening programme in the PROHTECT (*PROtection by Offering HPV TEsting on Cervico-vaginal specimens Trial*) studies. Main outcome measures included the compliance rate, the detection rate of CIN2+, and the concordance of HPV-test results between the self-sampled material and material sampled by the general practitioner.

Chapter 3 describes the findings of the PROHTECT 1 trial (n=27,163), performed between December 2006 – March 2007 within the setting of the organised screening programme. We compared the attendance rate of women who received a *lavage self-sampling device* (intervention group) to that of women who received a re-invitation for conventional cytology (recall control group). Furthermore, the yield of ≥CIN2/≥CIN3 in self-sampling responders was assessed. We showed that offering hrHPV testing on self-sampled vaginal material with a lavage self-sampling device to non-attendees significantly increases the attendance to the regular screening program, shows very good concordance with HPV test results on physician-taken scrapes in women with high-grade CIN, and proofed to be an effective way to detect high-grade CIN. The study demonstrated that offering self-sampling by sending a device for collecting cervico-vaginal specimens for high-risk HPV testing to women who did not attend regular screening is a feasible and effective method of increasing coverage in a screening programme. The response rate and the yield of high-grade lesions supported implementation of this method for women, not responding to an invitation for regular screening.

Chapter 4 describes the findings of the PROHTECT 2 trial (n=25,822), performed between December 2007 – March 2008 within the setting of the organised screening programme. In this trial, we evaluated the use of *a brush-based vaginal self-sampling device* in non-attendees of the cervical screening program for response rate, compliance to follow-up, concordance of HPV test results between physician-taken cervical scrapes and vaginal self-samples and CIN2+/CIN3+ yield. The same outcome parameters were assessed as in PROHTECT-1. Also this study demonstrated that offering self-sampling by sending a brush based device for collecting cervico-vaginal specimens for high-risk HPV testing to women who did not attend regular screening is a feasible and effective method of increasing coverage in a screening programme. However although the percentage of HPV positive women was lower than that of women using a lavage based device, the yield of CIN2, /CIN3 and cervical cancer was similar. The total yield of DNA sampled by the brush was 3x lower than that of the lavage based device.

In **Chapter 5** we compared the yield of CIN2+, CIN3+ and cervical cancer in the self-sampling responder women from the pooled PROHTECT studies (~30% of the non-responder women) who originally did not react to an invitation for regular cervical screening. Moreover we analysed the yield of CIN2+, CIN3+ and CxCa in the HPV responding group of the non-responders in relation to ethnicity and age of the women.

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Women, who did not react upon an invitation for regular screening (non-responder women) respond better to sending a device for self-collection of cervico-vaginal material for HPV testing (~30%) than to a reïnvitation for making a smear by a physician (11%). The relative risks for CIN2+ (1.6 (95%CI 1.4-1.9%) and CIN3+ (95%CI 1.5-2.1%) was significantly higher in the HPV responders in the non-responder group than in the regular responders of the cervical screening programme. From the non-reponder women,who react upon self-sampling devices those with the poorest screening history proved to have the highest risk for CIN2+, CIN3 and cervical carcinomas. Interestingly, independent of age and ethnicity, the self-sampling approach targets better the non-responder women who have never been screened and have the highest risk for (precursor lesions of) cervical cancer than women who were underscreened.

Women treated for CIN2+ are better followed by HPV testing in combination with cytology than by sole cytology. Moreover it is well known that women with HPV 16 infection have the highest risk for cervical cancer.(12;29;31) and that HPV testing. To further substantiate this we wondered whether in women treated for high grade CIN recurrence of high grade CIN was associated with certain HPV genotypes. In **Chapter 6** we analysed the presence of HPV genotypes in relation to recurrence of high grade CIN in women who underwent treatment for CIN2+ lesions. . We found that the post-treatment CIN3 rate is increased in HPV16-positive women treated for CIN3.

Finally, in **Chapter 7** we put the data from the chapters into perspective and describe in more detail our findings in relation to possible implementation of self-sampling for hrHPV testing in primary cervical screening.

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Chapter 2

Cytology history preceding cervical cancer diagnosis: a regional analysis of 286 cases

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Abstract

Background Despite programmed screening in The Netherlands, the decrease in incidence of cervical carcinoma lags behind. We analysed screening preceding carcinoma cases, timeliness in case of follow-up and FIGO stages as efficiency parameters for screening.

Methods We analyzed 286 women with cervical cancer between 2005–2007 for cytology history preceding carcinoma, hierarchically arranging cytology history (if present) into three groups: 'screened', 'work-up' and 'underscreened', (>6 yrs prior to diagnosis). For screen- and work-up smears we analyzed timeliness. FIGO stage were measured in relation to cytology history.

Results 99/286 (36.7%) women with cervical carcinoma were screened preceding the diagnosis. Delayed time-intervals in case of abnormal cytology was 43.5% for BMD and 38.0% for >BMD (p=0.51). 104/286 (36.4%) women were underscreened and 73/286 (25.5%) were unscreened. Advanced carcinoma or FIGO stage $\geq$2B in screened women was 16.0% versus 48.7% in work-up, underscreened or unscreened (p<0.001).

Conclusion Women with cervical cancer are underscreened and have poor timeliness in case of abnormal cytology. Being un- or underscreened correlates significantly with higher cervical cancer stages, especially in older women (aged $\geq$49 years; p<0.001). Improvement of attendancy is needed to meet the standards of quality for screening programmes.

Introduction

Cervical cancer is preceded by well defined premalignant lesions which can be identified by detecting abnormal cells in Papanicolaou (Pap) smear. Cervical screening by cytology with adequate treatment have resulted in a decrease in incidence and mortality of cervical carcinoma.(113;114) In the Dutch screening programme women aged 30-60 years are invited every 5 years, 7 times a lifetime. Modelling, prior to the introduction of the Dutch cervical screening programme, predicted a decrease in cervical carcinoma by approximately 75%, assuming full coverage(44), within the range mentioned in other studies.(4;115)

Coverage of the screening programme is currently 77%.(50) 65% of women attend the screening programme after an invitation, referred to as smears made *inside the screening programme* and 12% reflects smears made *outside the screening programme* (opportunistic smears). Approximately 23% of the invited women will not be screened at all.(85) Collectively, the effect on carcinoma incidence through these two modes of screening will be lower than modelled for the programme since full coverage is not attained. Moreover, the non-participating fraction of women (referred to as *non-attendees*) has a higher risk for cervical carcinoma than average, thus further decreasing the effectiveness of a programme in reducing carcinoma incidence.(116) Earlier studies have shown that 40-50% of the women diagnosed with cervical cancer are in the non-compliance group.(55;117)

Here we analysed 286 women with cervical carcinoma from the region Noord-Holland/Flevoland in the Netherlands diagnosed between 2005-2007 We analysed the relationship between the FIGO stage of the detected carcinoma and the associated screen status. In addition, we analysed whether the smear was made within or outside the screening programme, and the compliance for referral to the gynaecologist in case of an abnormal smear.

Methods

Data of regional carcinoma cases from PALGA

All cytological and histological results carried out in The Netherlands are excerpted in the Pathological National Automated Archive (PALGA), a centralised database. Since 1991, coverage is at least 95%.(118) We linked patient records based on identity of the encrypted first four letters of the maiden name and date of birth. The 'twinning-rate' is estimated to be around 2% per record.(119)

In total, our query in PALGA yielded 337,830 numbers of smears of which 334 cases (0.10%) with index-diagnosis histologically-confirmed cervical carcinoma in the years 2005-2007, and living in one region of the Netherlands. Groups of records presumably belonging to a single person were 'eyeballed' (checking every case manually) to filter out administrative twins by checking domicile, initials, and apparent inconsistencies in clinical history (n=48). This left 307,298 numbers of smears, of which 286 (0.09%) women were diagnosed with histologically-confirmed cervical carcinoma. Cervical smears were registered as either *inside*- or *outside* the screening programme. This study was approved by the ethical committee of PALGA (Pathological National Automated Archive).

Definition of 'cytology history' in this analysis

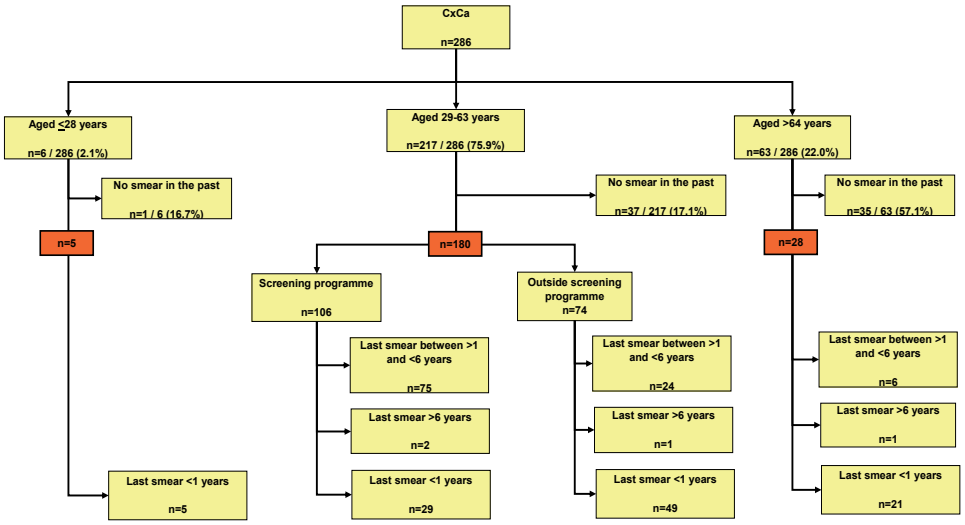
Screening histories of the 286 cases of carcinoma from PALGA database were analyzed for presence or absence of cytology history. For each woman, we took the moment with cervical carcinoma-diagnosis (t=0). From this point, we did a retrospective research in the preceding time period to determine their last cytological examination. Three timeframes were defined, 'unscreened' (no smear at all prior to the diagnosis of cervical carcinoma or only work-up smear prior to the diagnosis), 'underscreened' (last smear taken >6 years prior to the diagnosis – the work-up smear not included) or 'screened' (smear taken between 1 year and ≤6 years prior to the diagnosis). We defined cytology obtained less than 1 year preceding the diagnosis as a 'work-up smear'.

The choice of this time-period is based on the once-every-5-years-invitation of the Dutch screening programme protocol, which means that all women between 30 and 60 years of age are invited for programmed screening every 5 years, which is sent for free of charge for cytology. In relation to this interval, we have defined in our analyses the screening episode with an interval period of 6 years prior to the diagnosis of cancer until 1 year prior to the diagnosis. If a smear is detected in the database within this period, we consider the women 'screened'. Our three defined timeframes were categorized hierarchical: first, a woman was considered "screened" if she had a smear taken in this 1 to ≤6 year period. Secondly, a woman was considered "underscreened" if she had a smear >6 years prior to the diagnosis, but not in the *screened period*. Thirdly, a woman was considered "unscreened" if she had only a work-up smear or had had no smear at all in the past. Because of this hierarchical categorization, the women with a smear in the *screened* period could also have smears in the period >6 years prior to the diagnosis, and/or a work-up smear. Furthermore, women categorized *underscreened* could also have work-up smear.

Age stratification was done in nine groups of 5 years (i.e. <29 years of age, 29-33 years of age, 34-38 years of age, 39-43 years of age, 44-48 years of age, 49-53 years of age, 54-58 years of age, 59-63 years of age, and >63 years of age). Subanalysis of smears was based on the mode (i.e., invitational or inside the *screening programme* or *outside the screening programme*). For this subanalysis of 'within the screening programme', we have chosen for upper age 63, since the Dutch screening programme invites women up to age 60 (with a cut-off at 63 due to a possible follow-up time) and for lower age 29 years since women can be invited from the age of 29 years onwards. The number of eligible women (29-63 years of age) was 217 women for this subanalysis with respect to the screening programme (Figure 1).

In case of multiple abnormal smears in the screening history (e.g. in case of repeat cytology after BMD), we accepted the first abnormal smear as starting point. If women had only multiple normal smear results in the *screened period*, we analyzed the time-interval between the last smear prior to the diagnosis and the time of diagnosis. All analyses were performed using SPSS 15.0 software.

Figure 1: Flowchart of women with cervical carcinoma (stratified by 3 age groups, mode of screening, and cytology history). The flowchart is based on non-hierarchical categorisation of the cytology history.



Definition of work-up smear in this analysis

Work-up smears for diagnosis were defined as all cases of cytology obtained <1 year prior to the diagnosis rather than 6 months, because we considered women with a ≥BMD preceded by BMD cytology in the period of 12 to 6 months prior to the diagnosis, to represent women who should have had a severe abnormality in the first smear and thus be representative for women with signs and symptoms of carcinoma. We have taken this into account plus allowing a few months delay in repeat. Thus, we end the period for work-up smears at 1 year prior to the diagnosis.

Screening programme in the Netherlands and eligibility

Women are invited in the Netherlands in the year they become 30 years of age. Actually, at the time of screening they may still be 29 years of age. Similarly: at the second invitation, women may be still 34 years of age.

Women with a normal smear results will be invited again in the next screening round. Women with borderline/mild dyskaryosis (BMD) are advised to repeat the smear after 6 and 18 months. If at least one of the repeated cytology smears is read as BMD or worse, the woman will

be referred to a gynaecologist for colposcopy. Women with >BMD are referred immediately to a gynaecologist. Women with inadequate smears (not suitable for diagnosis) are advised to repeat the test after 6 weeks.

Time-interval between abnormal smear and diagnosis of cervical carcinoma

Among these women with cervical carcinoma we analyzed the time-interval between the first abnormal smear cytology and the histologically confirmed diagnosis. For timeliness, women were categorized as "not delayed" or "delay in diagnosis" (see Table 1 for definitions of timeframes). Smears with BMD that led to the histologically confirmed diagnosis ≤24 months, were considered as "not delayed". For smears with >BMD, timeliness was set on ≤6 months, as described by Bos et al.(55) For time-interval computations, all women with a smear taken up to 6 years (-2192 days) prior the diagnosis, were included in these analyses (thus encompassing both the screened group as the work-up group: n=195). Two cases with inadequate smears without follow-up were excluded, leaving a total of 193. After tabulating smear results, the time-interval was categorized in 2 periods: *not delayed* and *delay in diagnosis*.

Table 1: overview of the definitions in this manuscript: *screen smear (screening episode), work-up smear, diagnosis delay in diagnosis and screening interval.*

Definition	***Description***
Screen smear[1]	Smear taken between 1 year and ≤6 years preceding to diagnosis
Work-up smear	Smear taken maximum 1 year preceding to diagnosis
screening interval Dutch programme	Once every 5 years (between the age 30-60 years)
"delay" in diagnosis	***Interval between last smear and diagnosis***
Delay for borderline mild dyskaryosis (BMD	Cytology> 18 months preceding to diagnosis
Delay for worse than BMD (>BMD)	Cytology > 6 months preceding to diagnosis

[1] *screen smear* : smear taken in this period is considered as "screened in *screen period* or *screen episode*".

FIGO stage of cervical carcinoma related to cytology history and mode of screening

The FIGO stage of cervical carcinoma was related to the cytology history (i.e. presence or absence of a smear). Absence was defined as having no smear in the *screened period*. Furthermore, we also analysed the FIGO stage in relation to the cytology result of women with a

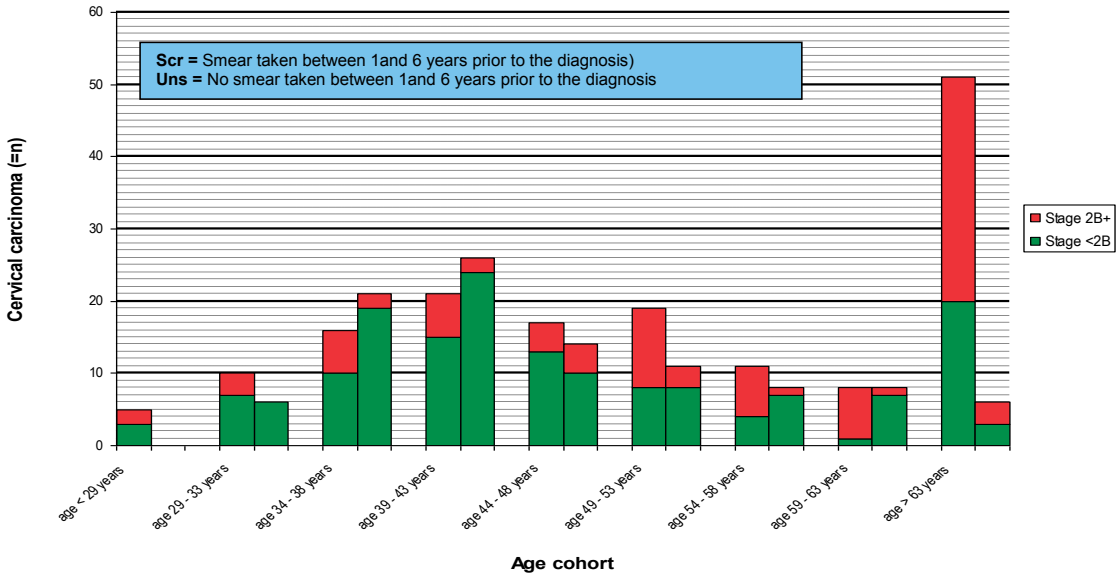
smear taken in the *screened period* allowing insight in whether an inadequate cytology advice results in a later detection of carcinoma. Both types of analyses were stratified by age eligibility for screening invitation, or other ages. These analyses were also stratified by age (see Figure 2). The analyses were done with the Fisher's exact test.

Results

Cases cervical carcinoma

The final query result from PALGA for the years 2005-2007 yielded 286 cases after excluding 48 cases due to double counting twinning, too late a diagnosis (i.e. in 2008), carcinoma of endometrial origin or metastasis. Age ranged between 25 and 93 years old (mean age: 50.8 years). We divided the remaining 286 women into an eligible group (n=217, aged 29-63) for receiving an invitation within the context of a screening programme, and a non-eligible group (i.e., aged <29 years (n=6), or >63 years (n=63), for falling outside invitational cohorts. The number of women without any smear in the period preceding the diagnosis was 1/6 (16.7%), 37/217 (17.1%), and 35/63 (55.6%) for women aged 29 years, 29–63 years, and >63 years, respectively (n=217; see Figure 1).

Figure 2: Women with histologically confirmed cervical carcinoma; the FIGO stage in relation to their screen smear, stratified by age cohort. Each age cohort contains two columns, of which the left columns presents women without a smear in the screened period (1 year - ≤6 years preceding diagnosis), and the right columns presents smear taken within this period.



Cytology preceding carcinoma cases: un(der)screened, screened or work-up smears

The hierarchically categorized table 2 shows that the number of women in the screened period is 105/286 (36.7%). In addition 18/286 women (6.3%) were *underscreened* (i.e., no smear between 1 year and ≤6 years). Furthermore, 90/286 (31.5%) only had a work-up smear, and, 73/286 women (25.5%) had no smear at all prior to the histologically confirmed diagnosis. The latter two groups represents the group of *unscreened* women. As can be seen in the one but lowest row of table 2, a subanalysis for women eligible for screening programme invitation, showed that 180/217 (99+16+65) women (82.9%) had at least a smear anytime preceding the diagnosis. This percentage was composed of 45.6% for 'screened' and 37.3% (7.4%+30.0%) for 'underscreened' and work-up smear. A total of 37/217 (17.1%) were 'unscreened', and had also no work-up smear prior to the diagnosis.

Table 2: The cytology history (hierarchical ordered into smear taken between 1yr – 6 yrs, smear >6 yrs, <1 yr or no smear) of women with histologically confirmed cervical cancer diagnosis between 2005 – 2007, stratified by age-cohort.

	Screen smear (i.e., between 1yr – 6 yrs)	**Smear taken >6 years**	**Smear taken <1 year †**	**No smear**	**Total**
Age cohort	N (% of row totals)	N (% of row totals)	N (% of row totals)		N (% of row totals)
≤ 28 years	-	-	5 (83.3%)	1 (16.7%)	6 (2.1%)
29-33 years	6 (30.0%)	-	11 (55.0%)	3 (15.0%)	20 (7.0%)
34-38 years	23 (56.1%)	-	12 (29.3%)	6 (14.6%)	41 (14.3%)
39-43 years	28 (54.9%)	5 (9.8%)	10 (19.6%)	8 (15.7%)	51 (17.8%)
44-48 years	14 (43.8%)	3 (9.4%)	11 (34.4%)	4 (12.5%)	32 (11.2%)
49-53 years	11 (32.4%)	3 (8.8%)	11 (32.3%)	9 (26.5%)	34 (11.9%)
54-58 years	9 (42.9%)	4 (19.0%)	6 (28.6%)	2 (9.5%)	21 (7.3%)
59-63 years	8 (44.4%)	1 (5.6%)	4 (22.2%)	5 (27.8%)	18 (6.3%)
≥ 64 years	6 (9.5%)	2 (3.2%)	20 (31.7%)	35 (55.6%)	63 (22.0%)
Total (aged 29-63 yrs)‡	99 (45.6%)	16 (7.4%)	65 (30.0%)	37 (17.1%)	217 (100%)
Total	105 (36.7%)	18 (6.3%)	90 (31.5%)	73 (25.5%)	286 (100%)

† smear taken <1 year prior to the diagnosis is considered as work-up smear for diagnosis

‡ women 29-63 years of age with non-attendancy possibility in the previous programmed screening round.

Cytology results in relation to cytology history

Table 3 shows the cytology results of historical smears. Interestingly, 69 of 105 women (63.8%) who were screened between 1 year - ≤6 years, had a normal or inadequate (n=2) cytology respectively. Furthermore, 15/105 (14.3%) had >BMD, but cervical cancer was diagnosed more than a year later suggestive of time delays. For women with only a work-up smear preceding the diagnosis, the results show a different pattern: >BMD was found in 75/90 women (83.3%). The results of cytology in the group of women screened >6 years prior to the diagnosis resembled the first group: 12/18 women (66.7%) had a normal cytology result, 5/18 women (27.8%) had BMD cytology result and only 1/18 (5.6%) had >BMD (Table 3).

In addition we have subdivided our group of underscreened women (n=18) with respect to the time interval between the last negative smear and the diagnosis of carcinoma into a subgroup with the last smear made 7-11 years for diagnosis and a group with the last smear made > 11 year for diagnosis. We could not find a significant difference in cytology result. However it should be realised that the number of women in the subgroups was very small for a meaningful analysis.

Table 3: Screen smears, smears taken more than 6 years ago and work-up smears in women with histologically confirmed cervical cancer diagnosis arranged according to cytology results.

Cytology	**Screen smear(i.e., between 1yr – 6 yrs)** Number (% of column totals)	**Smear taken >6 years** Number (% of column totals)	**Work-up smear (<1 year)** † Number (% of column totals)	**No smear at all**	**Total** Number (% of column total)
>BMD	15 (14.3%)	1 (5.6%)	75 (83.3%)	N/A	91 (31.8%)
BMD	21 (20%)	5 (27.8%)	11 (12.2%)	N/A	37 (12.9%)
Normal	67 (63.8%)	12 (66.7%)	2 (2.2%)	N/A	81 (28.3%)
Inadequate	2 (1.9%)	-	2 (2.2%)	N/A	4 (1.4%)
No smear	N/A	N/A	N/A	73 (100%)	73 (25.5%)
Total	105 (100%)	18 (100%)	90 (100%)	73 (100%)	286 (100%)

† smear taken <1 year prior to the diagnosis

Diagnosis of work-up smear in women with normal cytology results in the screened period

The normal (n=67), and inadequate (n=2) cytology results of women in the *screened* period, as shown in Table 3, does not exclude the possibility that these women had a work-up smear as well (since our analysis is hierarchical). Of the 69 women, 41 subsequently had >BMD (incl. the 2 women with inadequate cytology smear; 59.4%), and 9 had BMD (13.0%) as work-up smear. Furthermore, 2/69 (2.9%) and 1/69 (1.4%) had normal cytology or inadequate cytology, respectively. The remaining 16/69 women (23.2%) had no work-up smear. When further dividing these 53 work-up smears by the mode of screening ("within screening programme" and "outside screening programme"), the mode was not significantly different: 26/53 women (49.1%) with a work-up smear were found within screening programme versus 27/53 (50.9%) outside the programme.

In addition, we analysed possible work-up smears from women with >BMD and BMD in the screened period. Twelve of 15 women (80.0%) with >BMD in the screened period, again had >BMD, 1 woman (6.7%) had BMD and 2 women (13.3%) had no smear in the work-up for diagnosis period (not shown). Similarly, for women with BMD in the screened period, 13/21 (61.9%) had >BMD, 3/21 (14.3%) had BMD,1 woman (4.8%) had a normal cytology result and 4 women (19.0%) had no smear in the work-up period. Again, the distribution of the indication smear is equal for both groups (data not shown).

Screen smear and work-up smear in women with carcinoma in relation to mode of screening

For the subanalysis of cytology history in relation to the mode of screening (stratified by age-cohort), only women eligible (n=217) for programmed screening (aged 29–63 years), and having at least one smear $\leq$6 years before diagnosis (either screen smear or work-up smear. For definitions see Table 1), were selected (see Table 4). Screen smear and work-up smear are denoted separately. In addition, 14/18 women (77.8%) who were categorized as *underscreened*, also had a work-up smear and were therefore included. The remaining 4 women from this group were added to the 73 women without any smear prior to the diagnosis, since a work-up smear was lacking.

Only 99 of 217 women (45.6%) actually had a smear taken in the *screened period* ($p<0.001$), of whom 75/99 (75.8%) had a programmed smear and 24/99 (24.2%) had the smear taken outside the screening programme, which was statistically significant ($p<0.001$).

A further 78/217 (35.9%) women had a work-up smear only, divided between 29/78 (37.2%) (aged 29–63 years) for invitational cytology, and 49/78 (62.8%) outside the screening programme (see Table 4 and Figure 1). In total, 40/217 (18.4%) had no smear at all $\leq$6 years prior to the diagnosis.

Table 4: Screening mode of screen smear (i.e. between 1 yr – 6 yrs prior to the diagnosis) or work-up smear (< 1 yr prior to the diagnosis) subdivided into age cohorts of all 286 cervical carcinoma cases. The cases of women within age cohorts (29 – 63 years; n=217) of the Dutch screening programme indicated in bold.

	Screen smear (i.e., between 1yr – 6 yrs prior to diagnosis)		Work- up smear (i.e., <1 year prior to diagnosis)†		Cytology >6 years or no cytology preceding the diagnosis	Total
	Screening programme	Outside screening programme	Screening programme	Outside screening programme		
	Number	(% of row totals)	Number	(% of row totals)	Number (% of row total)	Number (% of column total)
≤ 28 years	-	-	-	5 (83.3%)	1 (16.7%)	6 (2.1%)
age 29 - 33 years	5 (25.0%)	1 (5.0%)	8 (40.0%)	3 (15.0%)	3 (15.0%)	20 (7.0%)
age 34 - 38 years	19 (46.3%)	4 (9.8%)	2 4.9%)	10 (24.4%)	6 (14.6%)	41 (14.3%)
age 39 - 43 years	19 (37.3%)	9 (17.6%)	8 (15.7%)	6 (11.8%)	9 (17.6%)	51 (17.8%)
age 44 - 48 years	11 (34.3%)	3 (9.4%)	3 (9.4%)	11 (34.4%)	5 (15.6%)	32 (11.2%)
age 49 - 53 years	7 (20.6%)	4 (11.8%)	3 (8.8%)	11 (32.4%)	9 (26.5%)	34 (11.9%)
age 54 - 58 years	6 (28.6%)	3 (14.3%)	2 (19.0%)	6 (28.6%)	4 (19.0%)	21 (7.3%)
age 59 - 63 years	8 (44.4%)	-	3 (16.7%)	2 (11.1%)	5 (27.8%)	18 (6.3%)
≥ 64 years	2 (3.2%)	4 (6.3%)	-	21 (33.3%)	36 (57.1%)	63 (22.0%)
Total screening age (29-63 yrs)	75 (34.6%)	24 (11.1%)	29 (13.4%)	49 (22.6%)	40 (18.4%)	217 (100%)
Total overall	77 (26.9%)	28 (9.8%)	29 (10.1%)	75 (26.2%)	77 (26.9%)	286 (100%)

† this includes women with a work-up smear, who also had a smear taken >6 years prior to the diagnosis, but not between ≤1yr – 6 yrs prior to the diagnosis: 14 of 18 (77,8%) women who were categorized in the period >6 years prior to the diagnosis had a work-up smear. The remaining 4 women are included in the category *No cytology <6 years prior to the diagnosis* along with women without any smear in the past (n=73).

Timeliness between first abnormal cytology smear and histologically confirmed carcinoma

Table 5 shows the timeliness between the first abnormal smear and histologically confirmed cervical cancer between 2005–2007 (stratified by cytology result: >BMD or BMD). We included all cytology taken up to 6 years prior to the diagnosis (thus both screen smear as well as work-up smear), which resulted in total 142 >BMD and 46 BMD cases. As shown previously in Table 3 shows that 15 and 21 women with cytology in the *screened period*, had >BMD, and BMD, respectively. Furthermore, cases with ≥BMD work-up smear of women who had had a normal or inadequate cytology smear in the *screened period* before diagnosis were included (41 and 9 of 69 women had >BMD, and BMD, respectively).

Table 5: Timeliness of abnormal (">BMD" or "BMD"), and normal smear preceding cervical cancer. All smear types (smear and work-up smears).

Cytology	Total	*Not delayed*	95% CI
>BMD[1]	142 (100%)	88 (62.0%)	53.5 – 70.0
BMD[2]	46 (100%)	26 (56.5%)	41.1 – 71.1
Total	188[3] (100%)	114 (60.6%)	53.3 – 67.7

1 maximum time-interval between the first abnormal smear and histologically confirmed CxCa was 6 months (a longer time-interval was considered as *delay in diagnosis*).

2 maximum time-interval between the first abnormal smear and histologically confirmed CxCa was 24 months (a longer time-interval was considered as *delay in diagnosis*).

3 In total 73 of 286 women had no smear at all preceding to diagnosis, leaving 213 cases with a smear. 25 of these 213 women had only normal cytology (n=23) or time-information was lacking (n=2), leaving 188 cases to analyse. This consisted of >BMD or BMD as follows: in the screen period 15 and 21 women had >BMD and BMD, respectively (see table 3). In the work-up period group, 75 and 11 women had >BMD and BMD, respectively. Furthermore, 41 and 9 women who had a normal smear result in the screen period, had >BMD and BMD, respectively, as work-up smear. Finally, for women who had a normal smear result in the underscreened period (n=12), 10 women had >BMD as work-up smear (The remaining 2 woman had normal smear result again). Another 1 - and 5 women had already >BMD and BMD, respectively, in the underscreened period (see Table 3). This results in a total of 142 >BMD, and 46 BMD (total n=188).

For timeliness, we divided the interval in 'not delayed' and 'delay in diagnosis' (see M&M for definitions). In the group of women with >BMD cytology smear, 88/142 (62.0%; 95% CI 53.5 – 70.0) showed no delay for diagnosis. In the group women with BMD, 26/46 (56.5%; 95% CI 41.1 – 71.1) had no delay in diagnosis. Delay in diagnosis for women with >BMD, and BMD were observed in 54/142 (38.0%), and 20/46 (43.5%), respectively. Overall, 60.6% of the women had no delay in diagnosis (95% CI 53.3 – 67.7). The difference in delay in diagnosis of between women with >BMD and BMD cytology smear was not significantly lower (p=0.51).

We also analyzed the work-up smears of women who had a *normal smear* (12 of 18 women; 66.7%) in the period >6 years before the diagnosis. In total 10 of these 12 women had >BMD (83.3%) and 2 of 12 women (16.7%) had again normal cytology result. The 6 of 18 women with ≥BMD result (1 woman with >BMD, and 5 with BMD) >6 year before diagnosis were, categorized as *delay in diagnosis*. Together with the 75 women with >BMD, and 11 women with BMD, the total >BMD, and BMD cases are 142 and 46, respectively. For the remaining 25 women timeliness was not applicable, since they had had either normal cytology (n=23) or time-information was lacking (n=2).

FIGO stage of cervical carcinoma in relation to cytology history

In Table 6a, we show the FIGO stage in relation to the cytology history. The FIGO stage was grouped into low grade (1A or 1B), borderline (2A-2B) and high grade (≥3A), because this grouping has consequences for therapy, and gives more relevant information about the relation between FIGO stage and screening history. FIGO stage information was available for 90.2% (258/286) of the cases, of which 196 and 62 in the group of women aged 29-63 years, and <29 years/>63 years respectively. Most women had no cytology smear taken in the *screened period* (158/258; 61.2%, 95% CI 55.3% - 67.2%), leaving 100 women with a smear taken in the *screened period* with a known FIGO stage in this group for analyses. In total 18 of 34 women (52.9%) with FIGO stage 1A had screen smear. Furthermore, 58 of 95 women (61.1%), 15 of 42 women (35.7%), and 3 of 25 women (12.0%) with a screen smear had 1B, 2A-2B, and 3A-4 FIGO stage, respectively.

When comparing the FIGO stage 1A to 1B, there is statistically no difference (p-value 0.424) in the percentage of screen smear. However, when we compare FIGO stage group 1B to 2A-2B, we notice a significant difference (p-value 0.009). Also a difference can be noticed when we compare the group 2A-2B to 3A-4 (p-value 0.047). Overall, the group with high-grade FIGO stage had significantly lower screen smear compared to the group with low grade FIGO stage (p<0.001; see Table 6a).

In the age group <29 years/>63 years, there were no significantly differences between the FIGO stage and having had a screen smear. Furthermore, we also analysed if a the differences between FIGO stage and two age-group (29-63 years vs <29 years/>63 years) was significant. The result showed that it was (p<0.001).

Table 6a: Women with a smear in screen smear period versus women without a smear in screen smear period, stratified according to FIGO stages, and eligibility for screening programme (aged 29-60 years, or otherwise). Inadequate tests are not included in this analyses.

	Cervical carcinoma	Screen smear (i.e., between 1yr – 6 yrs)†		Odds Ratio	
FIGO stage	Number	Number	Percentage within stage (95% CI)	Estimate (95% CI)	p-value
Aged 29 - 63 yrs					
Stage 1A	34	18	52.9% (35.1 – 70.2)	1.39 (0.58 – 3.30)	0.424 (stage 1A vs. stage 1B)
Stage 1B	95	58	61.1% (50.5 – 70.9)	0.35 (0.15 – 0.80)	0.009 (stage 1B vs. stage 2A-2B)
Stage 2A – 2B	42	15	35.7% (21.6 - 52.0)	0.24 (0.41 – 1.05)	0.047 (stage 2A-2B vs. stage 3A-4)
Stage 3A - 4	25	3	12.0% (2.5 – 31.2)	-	
Stage unknown	21	5	23.8% (8.2 – 47.2)	-	
Total	217	99	45.6% (38.9 – 52.5)		<0.001
Aged <29 yrs or >63 yrs					
Stage 1A	5	1	20.0% (0.5 – 71.6)	1.00 (0.39 – 73.56)	
Stage 1B	10	2	20.0% (2.5 – 55.6)	0.57 (0.55 – 8.18)	
Stage 2A – 2B	24	3	12.5% (2.7 – 32.4)	-	
Stage 3A - 4	23	-	-	-	
Stage unknown	7	-	-	-	
Total	69	6	8.7% (3.3 – 18.0)		0.211

† includes smears taken ≤1 year prior to the diagnosis, smears taken >6 years prior to the diagnosis, and women who never had a smear prior to the diagnosis.

Table 6b: Results of screen smear stratified according to FIGO stage. Of the 217 total cases in age group 29-63, 37 were without any smear leaving 180. In 17 cases FIGO stage could not assessed. Of the 69 in age group <29 years/>63 years, 36 were without any preceding smear leaving 33. In 6 cases no FIGO assessment was available.

	>BMD	BMD	Normal	Inadequate	Total	p-value
FIGO stage		Number	(% of column total)		Number (% of column total)	
Aged 29 - 63 years						
Stage 1A	12 (36.4%)	10 (30.3%)	11 (33.3%)	-	33 (18.3%)	0.151
Stage 1B	26 (30.2%)	15 (17.4%)	44 (51.2%)	1 (1.2%)	86 (47.8%)	0.620
Stage 2A – 2B	11 (39.3%)	4 14.3%)	12 (42.9%)	1 (3.6%)	28 (15.6%)	0.346
Stage 3A - 4	4 (25.0%)	5 (31.3%)	6 (37.5%)	1 (6.3%)	16 (8.9%)	
Stage unknown	13 (76.5%)	1 (5.9%)	3 (17.6%)	-	17 (9.4%)	
Total	66 (36.7%)	35 (19.4%)	76 (42.2%)	3 (1.7%)	180 (100%)	
Aged <29 or >63 years						
Stage 1A	2 (66.7%)	-	1 (33.3%)	-	3 (9.1%)	
Stage 1B	5 (71.4%)	2 (28.6%)	-	-	7 (21.2%)	
Stage 2A – 2B	9 (69.2%)	-	4 (30.8%)	-	13 (39.4%)	
Stage 3A - 4	4 (100%)	-	-	-	4 (12.1%)	
Stage unknown	5 (83.3%)	-	-	1 (16.7%)	6 (18.2%)	
Total	25 (75.8%)	2 (6.1%)	5 (15.2%)	1 (3.0%)	33 (100%)	

When we analysed FIGO stage of women with a *screen smear* (n=100), in relation to their cytology smear result (>BMD, BMD, or normal), no significant differences were found (see Table 6b). However, women aged 29/>63 years showed significantly more cytology lesions than women aged 29-63 years (p<0.001; data not shown).

We then analysed FIGO stage in relation to *screen smear* vs. otherwise per age strata (see Figure 3). We noticed a trend of more severe FIGO stage (FIGO stage ≥2B) among older women (aged 49 years and older; n=56/89; 62.9%) who had no smear in the *screened period*, compared to women who had their smear in the same period (n=8/33; 24.2%, p<0.001). In addition we analysed if a correlation could be found between the mode of screening and severe FIGO stage found among women (aged 34-63 years) who had their smear in the screened period. No statistically difference was found (p=0.822; data not shown).

Discussion

Our data show that only 36.7% (105/286) of women diagnosed with cervical carcinoma in the region Noord-Holland/Flevoland between 2005-2007 were appropriately *screened*. If restricted to eligible women for receiving an invitation for cervical screening (aged 29-63 years), this percentage was 45.6% (99/217). This finding is in agreement with a meta-analysis study, showing that about 46.2% women had a smear in the *screened period*.(120)

Further analysis showed that 67/105 women (63.8%) that were 'screened' had a normal cytology result (table 3). A meta-analysis study had an outcome of 29.3% of women with at least one normal cytology result in the same period.(120) However, 48 of the 67 (74.5%) women in our study subsequently had abnormal cytology (≥BMD) in their work-up smear, as assumed in previous modelling. Only 2 women (3.9%) had again a normal smear result in the work-up smear period. Even within the context of the limited reproducibility of cytology, this change from normal cytology to abnormal cytology in women with subsequent histologically proven cancer is likely to be caused by inappropriate sampling, processing or erroneous reading of the cytology.

Furthermore, our data show that 75/217 (34.5%) women from the eligible age cohorts, had been screened within the screening programme (see Table 4) in the appropriate period preceding the carcinoma diagnosis. From the residual 142, 29 were possibly 'screen detected' within the programme (13.4%), but were *underscreened.* The remaining 113/217 eligible women either did not have cytology ≤6 years preceding diagnosis (*underscreened period*; 40/217; 18.4%), or had obtained a smear outside the programme (73/217; 33.6%). These data shows that the detected histologically-confirmed carcinoma rate was higher among women who had not sufficiently attended organised screening (142/217) in the *screened period* (65.4% vs. 34.6%. p<0.001; See Table 4).

The results of timeliness between the first abnormal smear ≤6 years preceding the diagnosis and histologically confirmed diagnosis showed 62.0% (95% CI 53.5% - 70.0%) no *delay in diagnosis* among women with >BMD as cytology diagnosis and 56.5% (95% CI 41.1% – 71.1%) among women with BMD (p=0.51). This suggests that

significant improvements can be made in both compliance with repeats as well as redressing delays in workup.

The correlation between the FIGO stage and women with or without a cytology smear in *screened period* showed a difference for higher stages: a 1.7-fold higher cervical carcinoma stage (stage 2A-2B) was observed in carcinoma bearing women (aged 29-63 years) without cytology history, compared to women with 1B FIGO stage ($p=0.009$), while 1.4-fold higher cervical carcinoma stage (stage 3A-4) was found among women without screen smear, compared to women with screen smear and had 2A-2B FIGO stage ($p=0.047$). Furthermore, a trend can be seen in the relation between the higher stages and age of women. The older women (age $\geq$49 years) without a smear in the *screened period* showed significantly higher FIGO stages, compared to older women who had a smear in the same period ($p<0.001$). This indicates that non-screened *older* women are at higher risk for high stage cervical cancer.

In conclusion, our data shows that only 34.6% of all eligible women (aged 29-63 years) had participated in organised programmed screening between 1 year and $\leq$6 years prior the diagnosis (95% CI 28.2%–40.9%; $p<0.001$). Even if women screened outside the screening programme were included, the percentage would be 45.6%, which similar as reported in the literature.(120) Moreover, 67 woman had a normal screen smear followed by an abnormal smear in either the next round or in the work-up phase prior to carcinoma. These findings demonstrate both programme sensitivity as well as compliance need improvement. This may be achieved by implementing hrHPV testing as primary screening tool.(65;121-124)

There are several options to improve participation rate of organised screening programme. Hermens et al. have shown that women are more willing to attend in a screening programme when invitations are sent by their general practitioners in stead of the municipality.(82) Another option to influence the effectiveness of the screening programme, especially with regard to timeliness, is increased computerized controls to support physicians in controlling follow-up.(83) Finally, we recently showed that offering self-sampling for hrHPV DNA testing to non-attendees in the cervical screening programme is a feasible and effective approach, leading both to increased coverage and marked detection of CIN2+/CIN3+ lesions, particularly in women who did not attend the previous screening round.(125)

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Chapter 3

HPV testing on self collected cervicovaginal lavage specimens as screening method for women who do not attend cervical screening: cohort study

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ABSTRACT

Objective To determine whether offering self sampling of cervicovaginal material for high risk human papillomavirus (HPV) testing is an effective screening method for women who do not attend regular cervical screening programmes.

Design Cohort study (the PROHTECT trial).

Settings Noord-Holland and Flevoland regions of the Netherlands, December 2006 to December 2007, including 13 laboratories, gynaecologists, and more than 800 general practitioners.

Participants 28,073 women who had not responded to two invitations to the regular cervical screening programme: 27,792 women were assigned to the self-sampling group and invited to submit a self collected cervicovaginal sample for HPV testing; 281 were assigned to the recall control group and received a second re-invitation for conventional cytology.

Intervention Women with a positive result on the high risk HPV test on their self sample material were referred to their general practitioner. Women with abnormal results on cytology were referred for colposcopy. Women with normal results on cytology were re-evaluated after one year by cytology and high risk HPV testing and referred for colposcopy if either result was positive.

Main outcome measures Attendance rate in both groups and yield of cervical intraepithelial neoplasia grade II/III or worse (≥CIN II/≥CIN III) in self sampling responders.

Results The compliance rate in the self sampling group was significantly higher than in the control group (crude 26.6% v 16.4%, P<0.001; adjusted 27.5% v 16.6%, P<0.001). The number of detected ≥CIN II and ≥CIN III lesions in self sampling responders was 99 (1.3%) and 76 (1.0%), respectively. Self sampling responders who had not participated in the previous round of screening (43%) had increased relative risks of ≥CIN II (2.04, 95% confidence interval 1.27 to 3.28) and ≥CIN III (2.28, 1.31 to 3.96) compared with self sampling women who had been screened in the previous round (57%).

Conclusions Offering self sampling by sending a device for collecting cervicovaginal specimens for high risk HPV testing to women who did not attend regular screening is a feasible and effective method of increasing coverage in a screening programme. The response rate and the yield of high grade lesions support implementation of this method for such women.

Trial registration ISRCTN45527158.

INTRODUCTION

The introduction of organised cervical cancer screening programmes in Western countries has contributed to a decrease in incidence of and mortality from cervical cancer. Nevertheless, one major problem concerning the effectiveness of current cervical screening programmes remains non-attendance.[1-4]

Non-participating women (that is, non-attendees) are at increased risk of cervical cancer.56 Therefore, targeting non-attendees is important in achieving optimal protection from screening programmes. Offering self sampling of cervicovaginal material for screening has been suggested as a way of increasing screening compliance.[1,7,8]

Cytomorphological evaluation of self sampled cervicovaginal specimens for detection of high grade cervical lesions has been shown to be inferior compared with cervical samples obtained by a physician. Conversely, high risk HPV testing on self collected cervicovaginal samples had at least similar sensitivity for cervical intraepithelial neoplasia grades II/III or worse (≥CIN II/≥CIN III) compared with cytological reading of a corresponding cervical sample collected by a physician.[9-12]

METHODS

Patients and procedures

PROHTECT (protection by offering HPV testing on cervicovaginal specimens trial) is a cohort study within the setting of the Dutch population based cervical screening programme to assess the feasibility and efficacy of offering cervicovaginal lavage self sampling for high risk HPV testing to women who do not attend the regular screening programme. In the Dutch screening programme, women aged 30-60 are invited once every five years. Non-attendees living in the counties of Noord-Holland or Flevoland (n=28,073) who had received their screening invitation in 2005 were selected from the regional health council registry. In the screening programme women are asked to make an appointment to have a smear taken by their general practitioner or assistant. Opportunistic smears are disregarded but results are registered in the nationwide pathology database (PALGA). There is no charge for women participating in primary cervical screening, but the costs for the necessary follow-up in cases of abnormal cytology can be recovered from health insurance companies. A “non-attendee” was defined as a woman who neither responded to the regular invitation nor to a standard reminder after six months. Women with previous hysterectomy were excluded.

With a computerised random number generator non-attendees were assigned in a 99:1 ratio to either receive a kit (Delphi Screener (previous Pantarhei-Screener/Mermaid)) to collect cervicovaginal material for subsequent testing for high risk HPV hybrid capture II (self sampling group, n=27,792) or to receive a second recall for conventional cytology (recall control group, n=281). The Delphi Screener is a lavage device, designed to rinse the upper vagina and cervix and, in combination with HPV testing, has been shown to allow detection of similar high grade yields of CIN to those achieved in cervical smears collected by physicians.[9] The skewed ratio ensured

adequate power to detect a higher attendance rate in the self sampling versus second recall arm, while at the same time maximising the ≥CIN II/≥CIN III rate among self-sampling responders. The pre-randomised self sampling and recall cohorts were recruited from December 2006 to April 2007, and women were invited to respond within six weeks after the mailing. Only responses received up to December 2007 were scored for analysis.

All invited women were written to at their home address the week before to give notice of receipt of a second recall letter (recall control group) or a self sample kit (self sampling group). Women in the recall control group received an official second reminder to visit their general practitioner for conventional cytology, an explanatory letter, and an informed consent form. Women in the self sampling group received a self sample kit with an explanatory letter, a collection tube, instructions (written and drawn), an informed consent form, and a return box with the address of the testing laboratory. A telephone helpline and website providing information on the trial (www.hpvthuistest.nl) was available throughout the study.

HPV testing and follow-up algorithm Women in the self sampling group were asked to send the collection tube containing their cervicovaginal lavage specimen with the signed informed consent form to the laboratory for high risk HPV testing. Each specimen was tested with the hybrid capture II high risk HPV DNA method at the laboratory. The results of the confirmatory HPVsamples taken by general practitioner (see below) were also based on the hybrid capture II tests. On arrival in the laboratory, lavage specimens were concentrated by spinning down, removing all supernatant, and resuspending the pellet in universal collection medium. If no clear cell pellets were visible, samples were considered invalid for testing. In such cases, the woman was sent a second kit to repeat the self sampling at home. Valid samples were subjected to the hybrid capture II high risk HPV DNA test in an automated format on a rapid capture system), according to the manufacturer's instructions (Qiagen, Gaithersburg, MD, USA). This test is designed to detect high risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Results were expressed as relative light units per cut-off value (RLU/CO). Women with a positive result (RLU/CO ≥1) were instructed to visit their general practitioner for their doctor to take a cervical sample for cytology and confirmatory HPV testing in the regional laboratory serving the general practitioner. Women with abnormal cytology results (threshold borderline or mild dyskaryosis) were referred for colposcopy. Cytology of cervical smears taken by the physician was carried out in local laboratories, and results were reported according to the CISOE-A classification, the standard classification system for cytology in the Netherlands, which can easily be translated into the Bethesda classification.[10] In brief, on the basis of either squamous or columnar abnormalities, cytology results are categorised into three groups (normal, borderline or mild dyskaryosis, and moderate dyskaryosis or worse). Borderline or mild dyskaryosis corresponds in the Bethesda classification to ASCUS\LSIL. Endometrial abnormalities were excluded.

Independent of the HPV test result on the specimen taken by the physician, women with normal cytology results received advice for repeat testing (cytology and

HPV testing) after a year and were referred for colposcopy in case of a positive HPV or cytology test result (threshold ≥borderline or mild dyskaryosis). Women with negative results on both tests were referred back to the national screening programme as their risk of a clinically relevant lesion was considered too low to warrant referral for colposcopy.[11] Colposcopy directed biopsies were taken for histological examination from suspected areas on the cervix according to standard procedures in the Netherlands.[12,13] Histological examination was done in local pathology laboratories and specimens were classified as CIN 0 (that is, within
normal limits or including any non-neoplastic lesion such as inflammation, cyst, etc), I, II, or III, or as invasive cancer, according to international criteria.[14,15] On the basis of biopsy results, women were treated in accordance with the guidelines of the national screening programme.[16] Based on the cytology results, participating women in the recall control group were managed according to the guidelines of the national screening programme.[17]

Statistical analysis

Power calculation

For the power calculation, we estimated the population of non-attendees in the study area to be 45 000 in the year 2005. We further assumed 10% of these women would not be eligible and that the response rate in the self sampling group would be 15% higher than in the recall control group. The power calculation was based on our previous pilot,1 which showed compliance rates of 17.6% and 31.5% in the recall control and self sampling groups, respectively (two sided t test; α 0.05; 99:1 randomisation; power 0.99999). We chose a 99:1 randomisation to provide sufficient power to detect differences in compliance and to maximise the yield of ≥CIN II/≥CIN III in the self sampling cohort.

Response rate

All trial data were managed within a customised database. The self sampling response was counted on the basis of receipt of informed consent forms plus self-samples, and the response of the recall control group was counted on the basis of receipt of informed consent forms as well as through the nationwide network and registry of histopathology and cytopathology in the Netherlands (PALGA; Bunnik, Netherlands). For computing the response rate we included all women who responded actively within 12 months after their invitation to participate in this trial and compared response rates of the groups with $\chi 2$ test. In addition to the crude response rates, we also compared the response rates adjusted for women who during the study reported they were not eligible because of previous hysterectomy.

HPV prevalence and yield of high grade CIN and cervical cancer

Only self sampling material sent in within a year after the invitation was included in HPV prevalence analysis. The 18 month cumulative yields of ≥CIN II and ≥CIN III in women in the self sampling group who submitted a specimen were obtained through the

PALGA database. If necessary, physicians were contacted directly for additional clinical data.

Assessment of screening history self sampling responders

Programmed screening in the Netherlands involves seven cytology invitations during a lifetime to women aged 30-60—that is, every five years. The PALGA database provided the screening history of attendees in the two groups.[18] In analysing the screening history among the women who had been invited for a previous round of screening (that is, those aged ≥34), women were considered to have missed the previous screening round if a smear sample had not been taken within the past seven years.

Risks of ≥CIN II and ≥CIN III were calculated for women aged ≥34 who did not participate in the previous round relative to those who were screened in the previous round. The relative risks were adjusted for age by the Mantel-Haenszel method, with age stratified at 34-38, 39-43, 44-48, 49-53, 54-58, and ≥59. Women aged ≤33 could not have a screening history because of their age and were excluded. The association between the relative risks and age, as well as between the relative risks and screening history, were tested by the Mantel-Haenszel test of homogeneity.

RESULTS

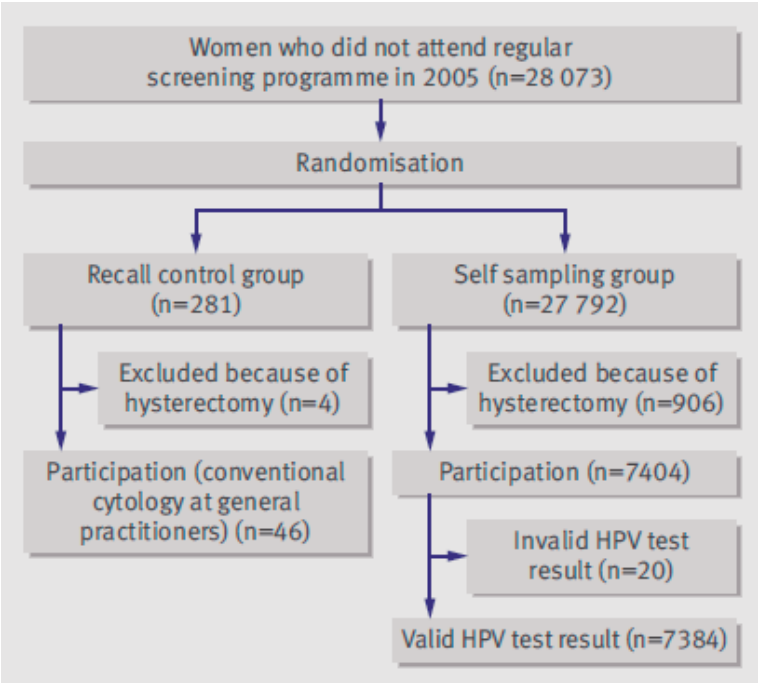
Characteristics of trial cohort

Figures 1 and 2 show the flow of women through the study. The public health database indicated that 28,073 women in the 2005 cohort who received an invitation to screening in the study area were registered as nonattendees, instead of the expected 45,000. Of these, 27,792 were allocated to the self sampling arm and 281 to the recall control arm. During the study 906 women (3.3%) in the self sampling group and four women (1.4%) in the recall control group reported having had a hysterectomy, leaving 26,886 eligible women in the self sampling group and 277 in the recall control group. There were no significant differences between the age distributions in both arms.

Participation rate

In the self sampling group, 7404 of 27 792 (26.6%) women sent a self sampled specimen for HPV testing and 51 (0.2%) decided to visit their general practitioner for conventional cervical cytology. When we adjusted for women who were not eligible because of hysterectomy, the percentage of women who responded by submitting a self sample was 27.5%. The self sampling response rate did not vary with age (Pearson $\chi 2=7.15$, df=6; P=0.307).

Figure 1: Study design for comparison of compliance rates between recall control group and self sampling group

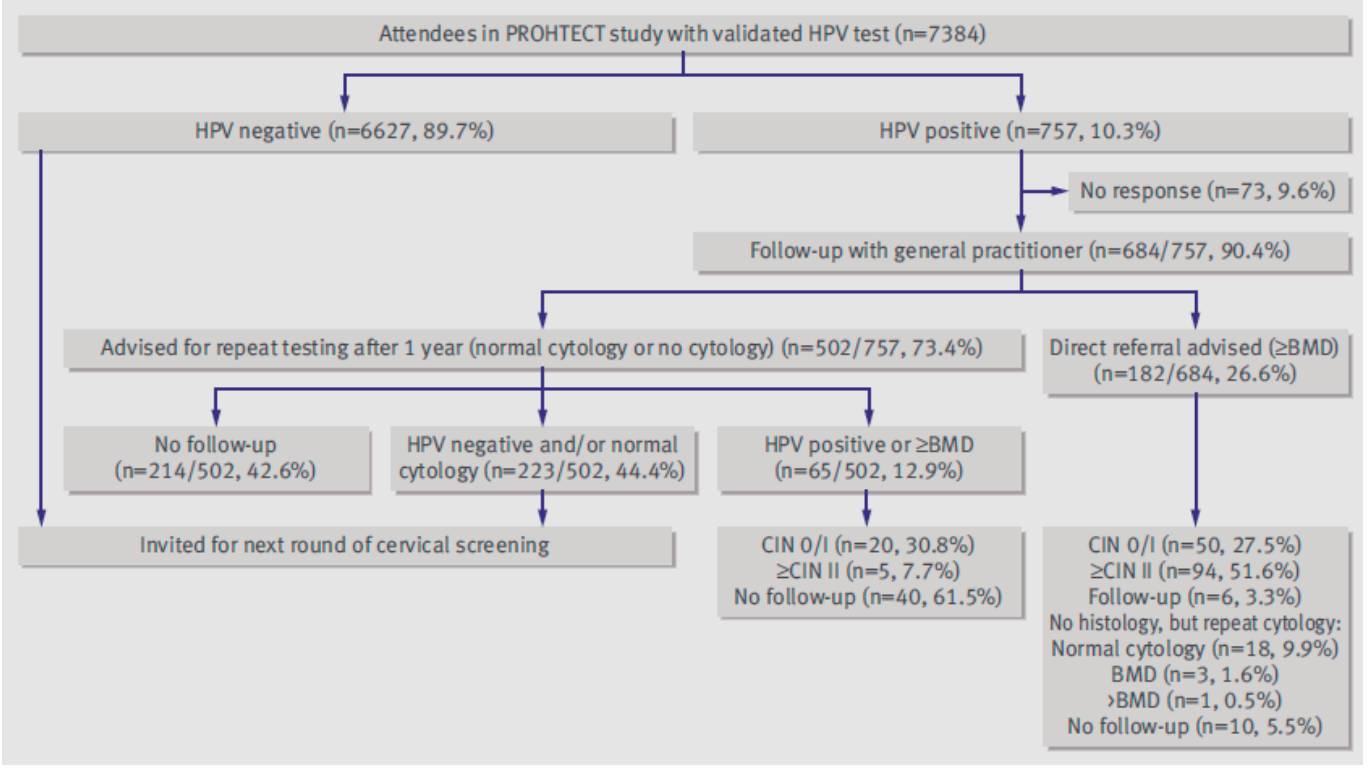


In the recall control group, 46 of 281 (16.4%) women visited their general practitioner for cervical cytology. After adjustment for non-eligibility, the response rate was 16.6% (46/277). Taken together, the difference in compliance rates between the groups was 10.2% (26.6% v 16.4%, 95% confidence interval 5.9% to 14.6%, P<0.001) or 10.9% (27.5% v 16.6%, 6.5% to 15.3%, P<0.001) after adjustment for those who were not eligible.

To evaluate whether screening history had an effect on participation in women allocated to self sampling, we compared the screening history of women in the recall control group (n=277) with women in the self sampling group (n=26,886). When we excluded women aged <33 because they could not have a screening history (49 in the recall control group and 3398 in the self sampling group), 228 eligible women were left in the recall control group and 22,988 in the self sampling group. There were 34 and 6,227 attendees aged ≥34, respectively. Of the 228 women in the recall control group, 61 (26.8%) had had a smear taken in the past seven years, compared with 5,967 (26.0%) of the 22,988 women in the self sampling group, indicating no significant differences.

We examined the effect of having no smear in the past seven years on attendance of women in the recall control group compared with the effect on attendance in the self sampling group. In the recall control group 9.0% (15/167) of the women responded to recall compared with 15.8% (2694/17 021) of the women in the self

Figure 2: Study design for evaluation of yield of ≥CIN II in women of self sampling group (BMD=borderline or mild dyskaryosis)



sampling group. Thus, the relative risk for participation of women without a smear in the past seven years in the self sampling group was 1.76 (1.09 to 2.86) compared with women who had no smear in the past seven years in the recall control group (P=0.016).

For the women who had had a smear in the past seven years, these proportions were 19/61 (31.1%) for the recall control group and 3533/5967 (59.2%) for the self sampling group, resulting in a relative risk of participation in the self sampling group of 1.90 (1.31to 2.76, P<0.001). As the relative risks of participation of women who had or had not had a smear in the past seven years did not differ (1.90 v 1.76, P=0.80), it seems unlikely that screening history introduced bias in the participation of women in self sampling in both groups.

HPV detection rate self sampling attendees

Of the 7404 women who submitted a self collected sample, 7384 (99.8%) had a valid hybrid capture II test result (fig 1) and 757 (10.3%) were positive for high risk HPV (fig 2). The percentage positive for HPV decreased with age (t=−6.77; P<0.001) until age 39-43, when a plateau was reached (t=−0.30; P=0.77).

Women who had positive results for high risk HPV were advised to visit their general practitioner for both conventional cytology and a second high risk HPV test on a cervical sample collected by the physician. A valid HPV result was recorded for 491, and 288 (58.7%) were positive for cervical HPV. Of the 203 with a negative result, 81 (39.9%) originally displayed hybrid capture II RLU/CO values <2 on the self collected sample. Conversely, only 21 (7.3%) with a positive result had a RLU/CO value <2 on the self sample.

Compliance with follow-up

Of the self sampling attendees with positive HPV results, 90.4% (684/757) complied with follow-up with their general practitioner. Of these 684, 437 (63.9%) followed the trial protocol and had both cytology and HPV test results, whereas 61 (8.9%) had only an HPV test and 186 (27.2%) had only a cytology test. Thus 623/757 (82%) women had cytological follow up. A total of 182 (26.6%) women had abnormal results on cytology, of whom 150 (82.4%) adhered to the direct referral advice for colposcopy. The 502 remaining women (73.4%) were advised have a repeat testing after a year on a sample collected by their general practitioner, and 287 (57.2%) complied. Based on a positive high risk HPV result or cytology result, or both, 65 (12.9%) women were advised to undergo colposcopy and 25 (38.5%) of them did so.

Yield of high grade CIN and cervical cancer

Among the 150 women with ≥borderline or mild dyskaryosis who visited a gynaecologist at baseline, 94 ≥CIN II lesions were detected, including five invasive carcinomas. Furthermore, five ≥CIN II lesions were detected among the 25 women who complied with the referral for colposcopy after repeat testing at one year. The cumulative 18 month yields of ≥CIN II and ≥CIN III in women with a positive HPV self sampling test were 1.3% (99/7384) and 1.0% (76/7384), respectively (table 1).

Table 1: Yields of ≥CIN II/III in women who carried out cervical self sampling (categorised by age)

Age (years)	No of women	No (%) with ≥CIN II	No (%) with ≥CIN III
≤ 33	1,157	29 (2.5%)	23 (2.0%)
34 – 38	1,497	30* (2.0%)	23* (1.5%)
39 – 43	1,266	10* (0.8%)	6* (0.5%)
44 – 48	1,139	11* (1.0%)	8* (0.7%)
49 – 53	918	9* (1.0%)	7* (0.8%)
54 – 58	825	6* (0.7%)	5* (0.6%)
≥ 59	582	4* (0.7%)	4* (0.7%)
Total	**7,384**	**99† (1.3%)**	**76† (1.0%)**
Total (excluding age ≤ 33)	**6,227**	**70† (1.1%)**	**53† (0.9%)**

Yield of high grade CIN and cervical cancer in relation to screening history

Among women aged ≥34, those who did not have a cervical smear taken at the previous round had more ≥CIN II (relative risk 2.04, 1.27 to 3.28, P=0.003; table 2) and more ≥CIN III (2.28, 1.31 to 3.96, P=0.003; table 3) than women who did have a cervical smear at the previous round. This association between screening history and cervical intraepithelial neoplasia was not related to age (P values Mantel-Haenszel test of homogeneity 0.639 for ≥CIN II and 0.515 for ≥CIN III). All five carcinomas were detected in women aged ≥34 who had not been screened at the previous round.

Table 2: Yield and risk of ≥CIN II in women aged ≥34 in relation to participation in previous round of screening (categorised by age)

Age (years)	Screened in previous round		Not screened in previous round		Rate of participation in previous round (95% CI)	Relative risk (95% CI) of ≥CIN II
	No of women	≥CIN II	No of women	≥CIN II		
34-38	809	13	688	17*	54.0 (51.5 to 56.6)	1.54 (0.75 to 3.14)
39-43	721	4	545	6*	57.0 (54.2 to 59.7)	1.98 (0.56 to 7.00)
44-48	684	3	455	8*	60.1 (57.2 to 62.9)	4.01 (1.07 to 15.03)
49-53	531	5	387	4*	57.8 (54.7 to 61.0)	1.10 (0.30 to 4.06)
54-58	463	2	362	4	56.1 (52.7 to 59.5)	2.56 (0.47 to 13.89)
≥59	325	—	257	4*	55.8 (51.8 to 59.9)	—
Total	3533	27	2694	43†	56.7 (55.5 to 58.0)	2.04 (1.26 to 3.28)

*Including one carcinoma.
†Including five carcinomas.

Table 3: Yield and risk of ≥CIN III in women aged ≥34 in relation to participation in previous round of screening (categorised by age)

Age (years)	Screened in previous round		Not screened in previous round		Rate of participation in previous round (95% CI)	Relative risk (95% CI) of ≥CIN III
	No of women	≥CIN III	No of women	≥CIN III		
34-38	809	11	688	12*	54.0 (51.5 to 56.6)	1.28 (0.57 to 2.89)
39-43	721	2	545	4*	57.0 (54.2 to 59.7)	2.65 (0.49 to 14.39)
44-48	684	2	455	6*	60.1 (57.2 to 62.9)	4.51 (0.91 to 22.25)
49-53	531	3	387	4*	57.8 (54.7 to 61.0)	1.83 (0.41 to 8.13)
54-58	463	1	362	4	56.1 (52.7 to 59.5)	5.12 (0.57 to 45.57)
≥59	325	—	257	4*	55.8 (51.8 to 59.9)	—
Total	3533	19	2694	34†	56.7 (55.5 to 58.0)	2.28 (1.31 to 3.96)

*Including one carcinoma.
†Including five carcinomas.

DISCUSSION

In the Netherlands in 2005, 65% of women attended the cervical screening programme (annual report of the regular screening programme, 2006, www.bevolkingsonderzoek.info/). By offering self sampling to non-attendees, and taking into account the 18% loss of cytology in the follow-up in this group, the real effect on attendance in the screening programme would be an extra 5.2% (6.3% (27.5% of 23%)*(100%−18%)).

The total attendance in the screening programme would then increase to 70.2% (5.2%+65%). Moreover, we showed that the cumulative incidence of ≥CIN II yield in our study was 1.3% (99/7384), while the CIN lesions found via regular screening programme in 2005 was 0.8% (data received from PALGA).

Screening history of non-attendees

The finding of a twofold and more than twofold relative risk of ≥CIN II and ≥CIN III, respectively, in self sampling women aged ≥34 who did not attend the previous screening round is in line with the assumption that background risk for ≥CIN II/≥CINIII is increased after women miss one screening round. In the self sampling group, the association between screening history and CIN was independent of age (P=0.639 for ≥CIN II and 0.515 for ≥CIN III).

Strengths and limitations

We did not include a recall control group for comparison of yield of ≥CIN II/≥CIN III with the self sampling group because data from our previous work indicated that non-attendees of the regular screening programme respond poorly to any repeat invitation letter.[1] Instead, we used a randomisation ratio of self sampling versus recall women in favour of maximising detection of ≥CIN II/≥CIN III in the self sampling group to allow an accurate assessment of the yield achieved by self sampling combined with HPV testing in non-attendees.

A potential bias in our attendance data could be that, unlike responders to a re-invitation for cytology, self sampling responders might have been more likely to respond for curiosity reasons, despite already being opportunistically screened before the study invitation. To address this we analysed the effect of screening history on participation via self sampling versus a second recall. When we took into account the screening history of women responders aged ≥34 in both arms, there was no indication that previously screened women would have a relatively higher preference for self sampling than women who were not screened within the past seven years.

Interestingly, self sampling responders showed high adherence to direct follow-up regimens, both at the general practitioner level (90.4%) and at the level of direct referral for colposcopy (94.5%). In size the latter is comparable with follow-up compliance of attendees of the Dutch screening programme (91%).[16] Compliance after repeat testing advice (59% of women attended after one year), however, was markedly lower than observed in regular screening attendees with similar advice (86%).[19] This

rather low return rate might be influenced by the fact that most of these women had previous normal cytology test results after a smear taken by a physician.

In 41% of the women who had an HPV test on both self and physician collected samples, a positive result for high risk HPV in the self sample could not be confirmed in the sample taken by the physician. Most of these discrepant test results were found in women with low hybrid capture IIRLU/CO values. In self sampled specimens more HPV infections of vaginal origin, including those of low risk HPV types, might be detected by hybrid capture II.[20-25] Even with a cut-off level of a positive result on hybrid capture II increased to RLU/CO ≥2, there are still discrepancies between positive results for HPV in the self sampled specimens and smears taken by the general practitioner. In that case the total number of HPV positive cases would decrease from 757 to 627, but we would miss six ≥CIN II lesions (two CIN II, and four ≥CIN III).

Interestingly, the yields of ≥CIN II and ≥CIN III in self sampling responders who attended the previous round and the yields in regular screening responders of the same age tested for high risk HPV by general primer 5+/6+ polymerase chain reaction (GP5+/6+ PCR) were identical (0.8% and 0.5%, respectively).[26,27] This strongly suggests that the ≥CIN II/≥CIN III sensitivity of HPV testing in self sampled
Cervico-vaginal material is not inferior to that of HPV testing on smears taken by a physician. This is in agreement with a recent meta-analysis that indicated that self sampling is as sensitive as physician obtained sampling to detect high risk HPV.[25]

Collectively, our data show that targeted efforts should be made to screen self sampling non-attendees who missed a previous screening round, given their increased risk of clinically relevant cervical disease. We have also shown that the chosen triage algorithm of a cytology test on a conventional smear after an HPV positive self sample is successful. A substantial subset of the 10% self sampling women who were positive for high risk HPV, however, seemed to have negative cytology results and be negative for high risk HPV at follow-up, which in practice resulted in a marked number of unnecessary visits to the general practitioner for these women. Therefore, alternative triage tools applicable to self sampled material should be considered to prevent redundant sampling by general practitioners. In this context, molecular methylation markers,[28] which are currently being investigated, are highly promising when applied to self sampled specimens. Furthermore, efforts are ongoing to improve liquid based cytological preparations of cervicovaginal lavage fluids for detecting abnormal cells.

Conclusions

It is feasible and effective to offer women who do not attend regular cervical screening programmes the choice of self sampling by sending a device for collecting cervico-vaginal specimens for high risk HPV testing. This should lead to increased coverage and marked detection of ≥CIN II/≥CIN III lesions, particularly in women who have not attended the previous round of screening. Implementation is likely to pay off immediately in terms of protecting a subset of non-attendees
known to be at increased risk of cervical cancer.

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Chapter 4

Experience with high-risk human papillomavirus testing on vaginal brush-based self-samples of non-attendees of the cervical screening program

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Abstract

We evaluated the effect of offering brush-based vaginal self-sampling for high-risk HPV (hrHPV) testing to non-attendees of the cervical screening program on response rate, compliance to follow-up and CIN2+/CIN3+ yield. In addition, concordance of hrHPV test results between physician-taken cervical scrapes and vaginal self-samples was determined.

26,409 non-attending women were randomly assigned to receive a vaginal brush device for hrHPV testing by HC2 (i.e., self-sampling group, n=26,145) or a re-invitation for regular cytology-based screening (i.e. recall control group, n=264). hrHPV-positive self-sampling responders were invited for a physician-taken scrape for cytology and blinded hrHPV testing. If cytology was abnormal, women were referred for colposcopy.

Response rate in the self-sampling group was significantly increased compared to the recall control group (30.8% versus 6.5%; $p<0.001$). The concordance rate between hrHPV detection in self-samples and corresponding physician-taken cervical scrape samples was 68.8%. Amongst women with CIN3+ and CIN2+, the concordance rates in hrHPV positivity between both samples were 95.5% and 93.8%, respectively. Adherence at baseline to cytology triage of hrHPV-positive self-sampling women (89.1%) and colposcopy referral of those with abnormal cytology (95.8%) was high. The CIN2+/CIN3+/carcinoma yields were 1.5%, 1.0%, and 0.1%, respectively, in self-sampling responders.

In conclusion, offering hrHPV testing on self-sampled vaginal material with a brush device to non-attendees significantly increases the attendance to the regular screening program, yields hrHPV test results that are in very good concordance with those of physician-taken scrapes in women with CIN2+/CIN3+, and is effective in detecting CIN2+/CIN3+.

Introduction

In the Netherlands, an organized cervical cancer screening program with a call and recall system, targeting women between 30 and 60 years of age every 5 year, is effective since 1996. Each year, 65% of the women attend the screening program.(1) Together with some opportunistic screening this contributes to an overall coverage for cervical screening of 77%, leaving 23 % of women unscreened.(1) The effectiveness of the screening program is strongly dependent of the degree of attendance.(2-5) Non-attendance is especially a problem in the youngest and oldest age groups of invitees (1;5). Women not attending the screening program have an increased risk of cervical carcinoma compared to attending women.(6) We have previously shown in the PROHTECT-1 trial that offering a self-sampling device for collecting cervico-vaginal lavage material for high-risk human papillomavirus (hrHPV) testing is a feasible and effective alternative for women not attending regular cytological screening, which improves coverage of the screening program significantly.(2;7)

Here, we present data of the PROHTECT-2 study, in which we evaluated the performance of brush-based vaginal self-sampling for hrHPV testing among non-attendees of the regular screening program. Outcome parameters were response rate, compliance to follow-up, concordance of hrHPV test results between vaginal self-samples and physician-taken cervical scrapes, and yield of cervical intraepithelial neoplasia grade 2 or 3, or worse (CIN2+ or CIN3+) within 18 months of follow-up.

Methods

Trial design

For PROHTECT-2, women were recruited who lived in the region Noord-Holland or Flevoland and, according to the database of the Regional Health Council, had not attended the organised cervical screening program in the year 2006 after the regular and reminder invitation. In that year, women who turned in their 30^{th}, 35^{th}, 40^{th}, 45^{th}, 50^{th}, 55^{th} or 60^{th} birth year were invited for the regular program. Women were invited for PROHTECT-2 between November, 2007 and March, 2008.

We randomised the women into a recall control arm and a self-sampling arm. The former did receive a second reminder invitation for regular cytology, while the latter received a self-sampling brush device (VibaBrush®, Rovers, Oss, The Netherlands) for hrHPV testing by the Hybrid Capture-2® method (HC2). Essentially the same trial design was used as for PROHTECT-1, in which HC2 test positive women were advised to visit a physician for a cervical scrape for cytology triage (see flowchart in Figure 1). All eligible women were sent a 1-week prior notice by surface mail to their home address to inform them about the study and alerting them on the possibility of either receiving a package for self-collection or a second re-invitation for regular cytology. After 1 week, women of the self-sampling group received a self-sample kit consisting of an explanatory letter with a brush for self-collection of a vaginal specimen, a collection vial containing 1.5 mL universal collection medium (Qiagen, Gaithersburg, MD, USA), written and drawn instructions, an informed consent form and a return envelope. Women of the control recall group received an official second reminder to visit their physician for

regular cytology, an explanatory letter and an informed consent form. A website and telephone desk providing information of the study were available throughout the study period (*http:// www.hpvthuistest.nl*).

We computed response rates in the recall control group and the self-sampling group and analysed in the self-sampling arm the CIN2+/CIN3+ yields within a period of 18 months after receipt of the hrHPV test result. Analyses were done via record tracking of individual cases as well as via query from the nationwide network and registry of histology and cytology database (PALGA; Bunnik, the Netherlands). To verify the follow-up data, the physician was contacted, if necessary.

Randomisation

To compare response rates, women were assigned to either a self-sampling group or a second re-invitation group for conventional cytology at a 99:1 ratio using computer's 'randomize number generator'. This skewed ratio was chosen to ensure adequate power to detect a difference in response rate between both study groups, but at the same time to maximize detection of CIN2+/CIN3+ rate among self-sampling responders.(7)

The study was approved by the Ministry of Public Health (no. 2006/01WBO), and registered in the trial register (*http://www.trialregister.nl/trialreg/admin /rctview.asp?TC=1851*) as *NTR1851*. All participating women gave written informed consent.

HPV testing of the self-sampled material

Women of the self-sampling group were asked to send the collection vial containing the self-sampled vaginal specimen together with the signed informed consent to the laboratory for hrHPV testing at the department of pathology, VU University medical center, Amsterdam. After visual inspection of the liquid samples in the lab these were tested by HC2 according to the manufacturer's protocol, in an automated format on a rapid capture system (RCS) (6;10;11) This test uses a high-risk HPV cocktail probe, which is designed to detect HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Results of HC2 were expressed as relative light units per cut-off value (RLU/CO). When no clear cell material was visible in the samples beta-globin PCR was performed first (8). In case of a negative beta-globin PCR test samples were considered invalid for HC2 testing. In this case, women received a second self-sampling kit with the request to repeat self-sampling at home.

Cytology reading

Cervical smears were read in local laboratories and results were reported according to the CISOE-A classification, the standard classification system for cytology in The Netherlands, which can easily be translated into the Bethesda classification.(9) For this analysis, cytology results on the basis of either squamous- or columnar abnormalities were grouped as normal, borderline or mild dyskaryosis (BMD;

corresponding to Bethesda ASCUS/LSIL), or moderate dyskaryosis or worse (>BMD; corresponding to ASC-H/HSIL or worse).

The follow-up triage of self-sampling group and control group

All responding women in the self-sampling group received a written test result and explanation by mail. Those who were hrHPV-negative were advised to await the next screening round invitation (i.e., 5 years after 2006). All women who were hrHPV-positive were advised to visit their physician for taking a scrape for cytology triage and blinded HPV testing. They were referred for colposcopy if the smear result was ≥BMD. In case of normal cytology they were re-invited after 1 year for a physician taken cervical scrape for cytology and hrHPV testing. Women with a positive cytology and/or hrHPV test result at that occasion were referred for colposcopy. In case a woman did not comply with the follow-up protocol at baseline or after 1 year, a reminder letter was sent to them with a copy to their physician. Women who had a double negative test result after 1 year, were advised to attend the next screening round.

Women responding in the recall control group received a cytology report of their physician-taken cervical specimen. Those with abnormal cytology were managed according to the guidelines of the national screening program (10;11). Endometrial abnormalities were excluded.

Outcome measures

The primary outcome measure of PROHTECT-2 was the response rate in both recall control and self-sampling arms. The time span for measuring attendance was 1 year from the moment the study responders received either the recall invitation or self-sampling device.

The secondary outcome measures included the prevalence of hrHPV among self-sampling responders, adherence to cytology triage and referral, and the number of histologically confirmed CIN2+/CIN3+ within a follow-up period of 18 months. Women with normal cytology and a hrHPV-negative cervical sample were not referred for colposcopy, because their risk of CIN2+ was considered that low that the medical ethics committee found it unethical to refer these women for colposcopy. In the analyses these women were assumed to have no CIN2+.

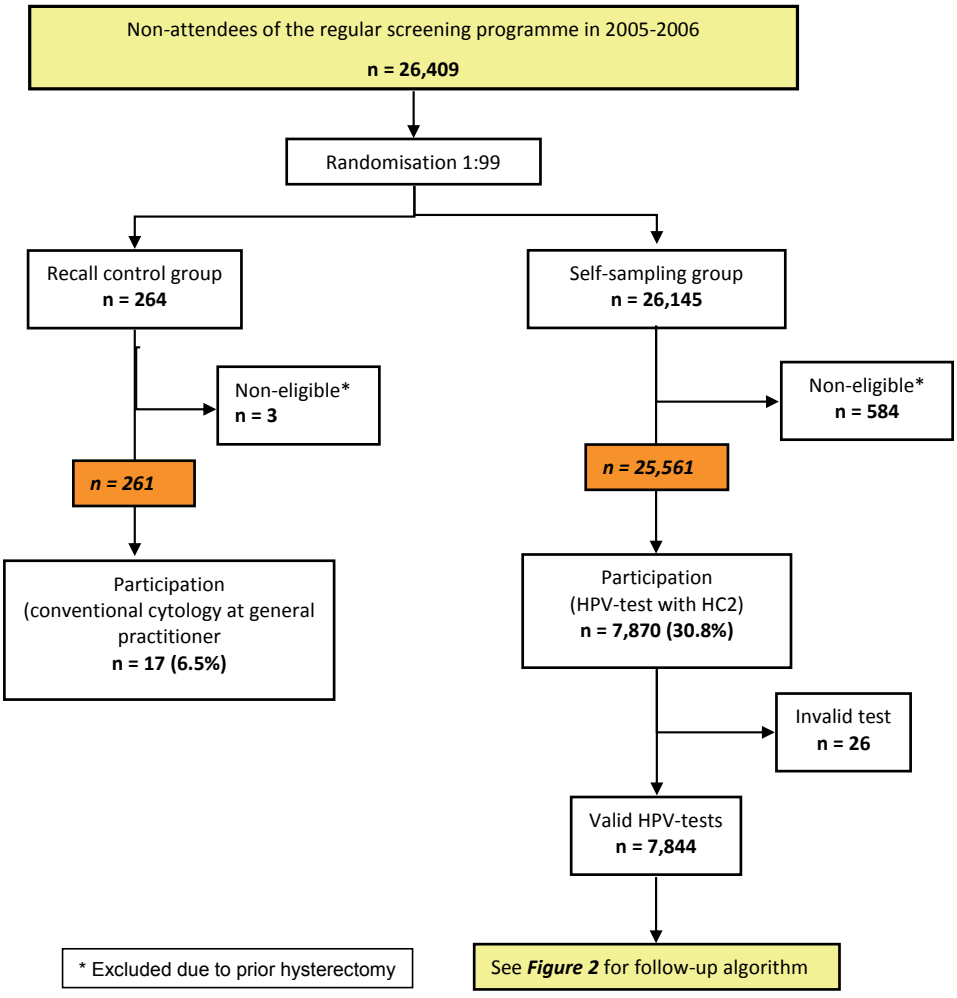
In addition, we assessed the concordance between hrHPV tests results on physician-taken smears versus self-sampled vaginal material of self-sample hrHPV-positive women and evaluated the CIN2+/CIN3+ outcome according to either HPV test result.

Results

Study cohort

A flowchart of the study design is given in Figure 1. A total of 26,409 non-attendees were eligible for inclusion in PROHTECT-2. After randomisation, 26,145 self-sampling kits were sent to women registered as non-attendees, and 264 non-attendees received a second reminder for regular cytology screening. 584 women in the self-sampling arm and 3 women in the control group did not respond to the study because they reported having had a hysterectomy or meanwhile passed away, leaving 25,561 women in the self-sampling arm and 261 women in the recall control arm, respectively. No statistically significant differences were found between the age distributions in both arms.

Figure 1: Study design for comparison of compliance rates between the recall control group and self- sampling group



Response rate

Of the self-sampling group, 7,870 women (30.8%; 95% CI 30.2% - 31.4%) submitted a self-sampled specimen. The response rate in the recall control group for cervical cytology was 17 out of 261 (6.5%; 95% CI 9.0% - 14.4%). The difference in response rate between the two study arms was statistically significant (χ^2 =71.77; p<0.01). In the self-sampling arm, young women (29-33 years) showed a significantly lower response rate than older women (χ^2 for linear trend=10.65; p<0.01), whereas no differences in response rate were found between women in the age strata from 34 to 63 years (χ^2 for linear trend=0.63; p=0.43).

hrHPV detection rate

Of the women who submitted a self-sampled specimen, 26 (0.3%) had an invalid hrHPV test result, leaving 7,844 women with a valid test (99.7%) (Figure 1). Among the latter, 652 (8.3%; Table 1) had a positive hrHPV test result. The percentage of hrHPV-positive women decreased with age from 15.6% in women of 29-33 years of age to 4.6% in women aged 59-63 years (χ^2 for linear trend=113.14; p<0.01). The proportion of hrHPV-positive women did not decrease with age in women of 44 years and older (χ^2 for linear trend=0.69; p=0.406).

Compliance to follow-up of hrHPV-positive women in self-sampling group

Seventy one of 652 (10.9%) hrHPV-positive women did not adhere to invitations for cytology triage testing, leaving 581 women (89.1%) with a cervical scrape taken by their physician. Of these, 28 women (4.8%) did not have a cervical smear, but only a hrHPV-test. Another 100 women (17.2%) had only a cytology result. The remaining 453 women (78.0%) followed the protocol and had both cytology and hrHPV-test results.

Of the 581 women with follow-up 192 (33.0%) had abnormal cytology (i.e. $\geq$BMD), of whom 184 (95.8%) adhered to the advice for direct referral to the gynaecologist for colposcopy. Another 8 women (4.2%) declined further follow-up (n=2), or did not comply to repeated advice for direct referral for colposcopy (n=6).

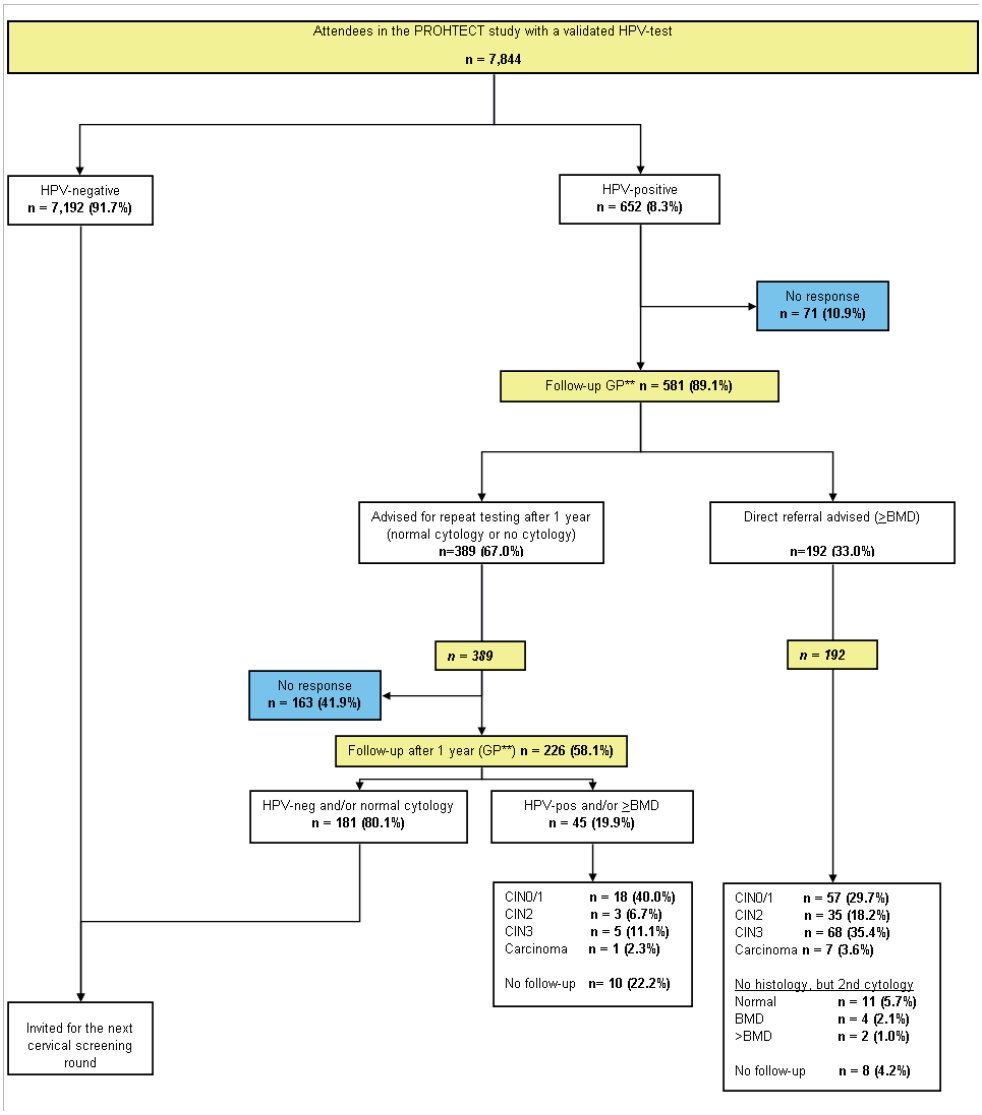
Of the 389 women without cytological abnormalities (including 28 women who had only a hrHPV test) 226 (58.1%) adhered to the follow-up protocol of repeat testing after 1 year. Sixty nine women (41.9%) declined further follow-up, and 94 women did not adhere to repeated advice for repeat testing at 1 year (in total 163 women). Amongst the 226 women with a repeat test after 1 year, 45 (19.9%) had cytological abnormalities and/or a hrHPV-positive test result. Twenty-seven (60.0%) of these women completed follow-up with histology (Figure 2).

Table 1: Response rate, hrHPV-prevalence and CIN2+/CIN3+/carcinoma yield in non-attendees to the regular screening program who responded by self-sampling, stratified by age

Age category	Total (n=)	Response rate (%)	HPV-pos	95% CI (HPV-pos)	CIN2+	CIN3+	Carcinoma
29-33 years	4,166	1,163 (27.9%)	182 (15.6%)	13.5% to 17.7%	42 (3.6%)	31 (2.7%)	2 (0.2%)
34-38 years	4,875	1,501 (30.8%)	164 (10.9%)	9.4% to 12.6%	35 (2.3%)	21 (1.4%)	3 (0.2%)
39-43 years	4,165	1,342 (32.2%)	102 (7.6%)	6.2% to 9.0%	16 (1.2%)	11 (0.8%)	-
44-48 years	3,766	1,167 (31.0%)	67 (5.7%)	4.5% to 7.2%	16 (1.4%)	10 (0.9%)	3 (0.3%)
49-53 years	3,147	986 (31.3%)	48 (4.9%)	3.5% to 6.2%	3 (0.3%)	3 (0.3%)	-
54-58 years	2,275	811 (29.2%)	48 (5.9%)	4.3% to 7.5%	4 (0.5%)	3 (0.4%)	-
59-63 years	2,667	900 (33.7%)	41 (4.6%)	3.1% to 5.8%	3 (0.3%)	2 (0.2%)	-
Total	25,561	7,870 (30.8%)	652 (8.3%)*	7.7% to 8.9%*	119 (1.5%)*	81 (1.0%)*	8 (0.1%)*

* These percentages are based on 7,844 as denominator (7,870 minus the 26 inadequate HPV self-sampled material.)

Figure 2: Study design for evaluation of CIN2+ yield in women of the self-sampling group.



Cervical carcinoma, CIN3+ and CIN2+ yield among hrHPV-positive self-sampling responders

Among 185 self-sampling responders with ≥BMD who visited a gynaecologist at baseline 7 cervical squamous cell carcinomas (3.6%), 68 CIN3 (35.4%), and 35 CIN2 (18.2 %) lesions were detected. Most CIN2+ lesions were found in the youngest age group (29-33 years).

Of the 27 women who underwent colposcopy after one year, 1 had cervical carcinoma (3.8%), 5 had CIN3 (18.5%) and 3 had CIN2 (11.1%). All of these 9 women had abnormal cytology and those tested for hrHPV (n=3) were hrHPV positive. At baseline 8 of these 9 women had normal cytology and 1 had no cytology result. The 5 women with CIN3 and 1 woman with CIN2 had a hrHPV-positive physician-taken scrape, whereas 2 remaining women with CIN2 had an invalid baseline hrHPV test on the physician-taken scrape. Strikingly, in the woman with cervical cancer the hrHPV test performed on the physician taken smear at baseline was negative.

The cumulative 18-month CIN3+ and CIN2+ yields in women with a hrHPV-positive self-sampling test were 1.0% (81 of 7,844) and 1.5% (119 of 7,844), respectively (see Table 1 and Figure 2). After stratification into age groups, the CIN2+/CIN3+ yields appeared significantly higher in young women (aged 29-33 years) compared to older women (aged 34-63 years; CIN2+: 3.6% vs 1.1%, respectively; $p<0.001$, and for CIN3+: 2.7% vs 0.7%, respectively; $p<0.001$). Also, significant differences were found when comparing women aged 29-38 years to women of 39-63 years (CIN2+: 2.9% vs 0.8%, respectively; $p<0.01$; CIN3+: 2.0% vs 0.8%, respectively; $p<0.01$).

hrHPV test results of physician-taken cervical scrapes versus self-samples in relation to histological outcome

In total 481 out of 652 (73.8%) women with a hrHPV-positive vaginal self-sample test also had a hrHPV-test performed on their physician-taken cervical scrape at baseline. The mean time interval between the self-sampling test result and that of the physician-taken scrape at baseline was 76 days (median: 58 days). Three smears (0.6%) had invalid hrHPV results, leaving 478 samples (99.4%) with a valid test result. The concordance between the hrHPV test result of physician-taken cervical scrape and vaginal self-sample was 68.8% (329 out of 478 women) (95% CI 64.7% to 73.0%; see Table 2).

Table 2: HPV test results on physician-taken cervical scrapes of 652 women with hrHPV-positive self-samples in relation to histological outcome

	≤ CIN 1	CIN 2	CIN 3	CxCa	No hist fup	Total
HPV-positive	50 (80.6%)	27 (90.0%)	61 (96.8%)	3 (75.0%)	188 (58.9%)	329 (68.8%)
HPV-negative	12 (19.4%)	3 (10.0%)	2 (3.2%)	1 (25.0%)	131 (41.1%)	149 (31.2%)
Invalid HPV test*	0	2	-	-	1	3
No HPV-test performed**	13	6	10	4	138	171
Total	**75 (100%)**	**38 (100%)**	**73 (100%)**	**8 (100%)**	**458 (100%)**	**652 (100%)**

* Samples inadequate for HPV testing and **women without an HPV test on physician-taken cervical scrape are not included in the given percentages

The mean time interval between the self-sampling test result and that of the physician-taken scrape at baseline of the 329 women with an hrHPV positive physician sample was 68 days (range 6-332 days) with 28 women (8.5%) having their physician-taken sample ≥4 months after the self-sampling result. In contrast for the 149 women with a hrHPV-negative physician-taken sample the mean time interval between the self-sampling test result and the physician-taken scrape at baseline was 92 days (range 16-337 days) with 38 women (25.5%) having their physician-taken scrape taken ≥4 months ($p<0.001$). This longer time interval in women with an hrHPV-negative physician-taken scrape suggest that these women have cleared their HPV infection in the time interval between self-sampling and the physician-taken scrape. In women with CIN2+ and CIN3+ the concordance rate in hrHPV-positivity between both samples was very high (CIN2+: 91/97 (93.8%), and CIN3+: 64/67 (95.5%; see Table 2).

The histological outcome in relation to the hrHPV test result on the physician-taken scrapes in women with hrHPV positive self-samples at baseline is shown in Table 2. Of the 8 women with carcinoma, 4 (50.0%) had a hrHPV-test performed on a physician-taken scrape, of which 3 (75.0%) were hrHPV-positive. This hrHPV-negative woman also had normal cytology at baseline and was detected by abnormal cytology after 1 year. Of the women with CIN3, 61 of 63 (96.8%) of the physician-taken scrapes were hrHPV-positive. Of the 30 cervical scrapes of women with CIN2, 27 (90.0%) were hrHPV-positive. 80.6% (50/62) of the physician-taken cervical scrapes of self-sample hrHPV-positive women with ≤CIN1, were hrHPV-positive as well. The remaining 188 women with hrHPV positive physician-taken scrapes had no histology follow-up data.

Discussion

In this study, we showed that offering a brush for vaginal self-sampling to non-attendees of the regular screening programme significantly increases the response rate

in the cervical screening programme compared to a repeat reminder for a physician-taken scrape. Together with the high 18 month yield of CIN2+ (1.5%;119 of 7844) and CIN3+ (1.0%;81 of 7,844) obtained following hrHPV HC2 testing of these samples, this indicates that vaginal self-sampling using a brush is an attractive approach to increase the effectiveness of the cervical screening program, both in terms of response rate and high-grade lesion yield.

The adherence of the responders with a positive hrHPV test to a cytology triage test was high (89.1%), and that to direct colposcopy referral after abnormal cytology even higher (95.8% at baseline). However, young (age 29-33 years) and older (age >53 years) women with hrHPV-positive self-sampled material showed a lower adherence to cytology triage compared to women aged 34-53 years. Such an age difference was also observed in previous studies on attendance to screening programs.(1;5;12) We noticed that sending reminders to hrHPV-positive women and their physicians explaining the consequences of the test result increased the adherence to cytology triage at baseline. Janerich et al. also showed that the use of patient reminder systems for adherence to follow-up procedures can greatly reduce the number of women with a delayed diagnosis of CIN2+.(12) Conversely, loss to follow-up after a one year repeat testing advice of women without abnormal cytology at baseline was substantial (58.1% of these women complied after one year). A similar compliance with one year follow-up (i.e. 57.4%) was obtained in the PROHTECT-1 study.(7) Therefore, we expect that the yield of CIN2+/CIN3+ lesions detected in this study is still underestimated. The positive effect on compliance of sending reminders to women and their physicians strongly argue for adding this approach in a recall system of these women.

Amongst all outcome measures analysed in this study only response rate in the self-sampling group and and hrHPV positivity differed slightly with those of PROHTECT-1. The self-sampling response rate was slightly higher (30.5% vs 27.4%) and hrHPV positivity somewhat lower (8.3% vs 10.3%) in this study compared to PROHTECT-1. We do not have a good explanation for these findings. However, the total yield of CIN2+ and CIN3+ among participated women did not differ (PROHTECT-1: 99/7,384 (1.3%) and PROHTECT-2: 119/7,844 (1.5%) for CIN2+, and PROHTECT-1: 76/7,384 (1.0%) and PROHTECT-2: 81/7,844 (1.0%) for CIN3+). This indicates that for the detection of high-grade CIN lesions and cervical carcinomas by hrHPV testing both self-sampling devices show good results. However, we noticed that the amount of cells collected by the brush self-sampler device is at least 3 times lower than obtained by the Delphi cervico-vaginal lavage self-sampler (data not shown). Together with the fact that brush samples primarily contain vaginal cells, this makes brush sampled material less suited for additional molecular tests for disease markers.

The overall concordance between hrHPV-positive physician-taken scrapes and self-samples at baseline was 68.8%. The hrHPV-negative results on the physician-taken scrapes may in part be explained by the presence of vaginal HPV infections detectable by HC2 (7). In addition, 25.5% of women with a hrHPV-negative physician-collected sample visited their physician for a cervical scrape at least 4 months after self-sampling, and might have cleared the hrHPV infection in between. By comparison, only 8.5%

women with a hrHPV-positive physician-obtained sample waited ≥4 months to visit their physician for a cervical scrape. Importantly, the concordance between hrHPV test results on physician-taken scrapes and self-sampled vaginal material of women with high-grade CIN or cervical cancer, and consequently a persistent HPV infection, was very high (>95% for CIN3+).

The strengths of this study are its large size and performance within the setting of the regular screening program. The data confirm those of the earlier study performed with a lavage self-sampler (PROHTECT-1) conducted in the same region.(7) In both studies, the response rate was approximately 30%, indicating that a similar proportion of non-attendees can be reached by offering hrHPV testing on self-sampled material collected by both devices. Even more important is that in both studies similar yields of high-grade CIN and cervical cancer were obtained.

The poor adherence to follow-up testing after 1 year is a weakness of this study (58.1%), especially when compared to the high compliance rate at baseline (89.1%). As pointed out before, this has a likely negative effect on CIN2+/CIN3+ yields. Striking was that even women with abnormal cytology after 1 year showed a relatively poor adherence to referral for colposcopy (60.0% vs. 95.8% at baseline). Although we do not have an explanation for this finding, we had the impression that better education of the women as well as physicians about the possible screening results might help to improve the compliance to visit the physician after 1 year.

Many studies, often performed on small numbers of women and with different self-sampling devices and hrHPV detection techniques have compared self- versus physician-sampling, though mostly at the level of HPV test performance rather than the yield of CIN2+/CIN3+.(13-16) Generally, a high level of concordance between the HPV test results on self- versus physician-taken samples was obtained.(13-14) As we have shown earlier offering HPV testing on self-sampled cervical material should not only be evaluated at the level of HPV test performance but also the level of CIN2+/CIN3+ yield.(16) In fact, the whole chain of self-sampling, HPV testing with a clinically validated test (17), follow-up of HPV-positive women and the CIN2+/CIN3+ yield of the referred women should be evaluated before self-sampling can be introduced in routine screening. Non-attendees of the regular screening program form an ideal group of women to test the performance of HPV self-sampling, not only because the prevalence of high-grade cervical lesions is higher than in women who participate in regular screening program(85), but also because there is no other effective alternative for non-attendees.

Together, the results of both PROHTECT-1 and PROHTECT-2 are so encouraging that efforts are warranted to study offering self-sampling as a more pleasant alternative for a physician-taken smear to women (age 30-60 years) invited for regular screening programs.

In summary, offering hrHPV testing on self-sampled vaginal material with a brush device to non-attendees of the regular screening program significantly increases the attendance to the regular screening program, results in HPV test results that are in good concordance with those on physician-taken scrapes in women with CIN2+/CIN3+ and is effective in detecting CIN2+/CIN3+.

Conflict of interest statement

CJLM Meijer, PJF Snijders, and DAM Heideman are shareholders of self-screen, a recent spin-off company of VUmc medical center. CJLM Meijer is on the scientific advisory board of Qiagen, the manufacturer of the Hybrid Capture-2® HPV test. All other authors declare that they have no conflict of interest. The sources of funding did not have any influence on the design and the analysis of the results.

Acknowledgments

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Chapter 5

Offering self-sampling for HPV testing to non-attendees of the cervical screening programme: characteristics of the responders

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Abstract

Background Self-sampling for high-risk HPV (hrHPV) testing is accepted by up to 30% of non-attendees to the regular cervical screening programme. Here, the yield of CIN2 or worse (≥CIN2) and CIN3 or worse (≥CIN3) of 15,274 HPV self-sampling responders amongst non-attendees was compared to that of 176,027 women participating in regular screening in the same period and in the same region. We also analysed which subpopulations amongst non-attendees are targeted by HPV self-sampling, and which characteristics relate to hrHPV prevalence and yield of ≥CIN2/≥CIN3.

Method Data from two consecutive self-sampling studies were pooled. ≥CIN2/≥CIN3 yields, screening history, age and ethnic status were retrieved from centralized pathology and screening databases, respectively. A logistic regression model was fitted to analyze method of invitation, ethnicity, age group, and screening history as predictors for response rate, hrHPV presence and ≥CIN2/≥CIN3 in non-attendees. For screening history analyses, women <34 years were excluded since it was the first screening round in their life.

Findings ≥CIN2/≥CIN3 yields of HPV self-sampling responders were higher than those of screening participants (≥CIN2: relative risk (RR)=1.6, 95%CI=1.4-1.9; ≥CIN3: RR=1.8, 95%CI=1.5-2.1 with relative risk values increasing with age (Test of Homogeneity: ≥CIN2: p=0.04; ≥CIN3: p=0.03).
Native Dutch non-attendees responded better than immigrants (32% versus 22%, p<0·001) and those screened in the previous round revealed a higher response than underscreened (i.e., previous smear taken >7 years ago) or never screened (34% versus 25%, p<0·001) women. Strikingly, amongst under- and never screened women aged ≥39 years, never screened women responded better (25% versus 23%, p<0·001). ≥CIN2 rates were higher among responding native Dutch women than immigrants (p<0·01), and higher in under-/never screened women than in women screened in the previous round (p<0·01).

Interpretation Offering hrHPV self-sampling increases the efficacy of the screening programme by targeting a substantial portion of non-attendees of all ethnic groups who have not regularly been screened and are at highest risk of ≥CIN2.

Introduction

Organised cervical screening programmes have reduced the incidence of and mortality from cervical cancer.(1-3) Non- or infrequent attendance is one of the main threats to the success of those screening programmes.(4) Targeting non-attendees is important because these women have an increased risk of cervical cancer.(5) Recently, we found that offering self-sampling for high-risk HPV (hrHPV) testing (further referred to as HPV self-sampling) to non-attendees is an effective approach for increasing screening coverage (PROHTECT studies).(6;7)

Nevertheless, it is still unknown which subpopulations of non-attendees, in terms of age, ethnicity and screening history, are targeted by HPV self-sampling. It is known that screening participation rates vary across ethnic populations.(8) Moreover, not being screened within previous screening intervals has been found to be associated with increased risks of cervical intraepithelial neoplasia (CIN) grades 2 and 3, and cervical cancer.(6;9-12)

Here, we used the pooled data from the two consecutive PROHTECT HPV self-sampling studies comprising a total of 52,447 non-attendees of the regular screening programme recruited from 230,509 women invited for cervical screening in the counties Noord-Holland and Flevoland in 2005 and 2006. First, we compared the yield of CIN2 or worse (≥CIN2) and CIN3 or worse (≥CIN3) of HPV self-sampling responders (n=15,274) with that of their counterparts participating in primary cytology-based screening (n=176,027). In addition, we analysed which subpopulations amongst non-attendees are targeted by HPV self-sampling, and how these characteristics relate to hrHPV prevalence and yield of ≥CIN2 and ≥CIN3.(6;7)

Methods

Study population

Non-attendees of the regular screening programme

All 54,482 women out of 230,509 invitees (aged 30-60 years) in the counties Noord-Holland and Flevoland who did not attend the cervical screening programme after two invitations in 2005 and 2006 were registered as screening non-attendees and were recruited to participate in the PROHTECT studies from December 2006 to March 2008.(6;7) In these studies, the effect of offering self-sampling for hrHPV DNA testing by Hybrid Capture 2 (HC2) on response rate and cumulative 18-month ≥CIN2/≥CIN3 yield was evaluated. Response rate was compared with women who received a second reminder for conventional cytology (recall control group). Written informed consent was provided by all women. The studies were approved by the Ministry of Public Health (no. 2006/01WBO) and registered as International Standard Randomized Controlled Trial, numbers ISRCTN45527158 (PROHTECT-1) and NTR1851 (PROHTECT-2). In the PROHTECT-1 study (non-attendees in 2005), self-sampling of a (cervico)vaginal specimen by a lavage-based device (Delphi®-Screener, Delphi-bioscience, The Netherlands) was

offered to 27,792 women (self-sampling group), and a second recall for conventional cytology was sent to another 281 women (recall control group).(6) In PROHTECT-2 (non-attendees in 2006) a brush-based self-sampling device (VibaBrush®, Rovers Medical Devices, The Netherlands) was offered to 26,145 women, whereas 264 women received a second recall for cytology.(7) Further study details have been described before(6;13;14). Apart from the self-sampling method, both PROHTECT studies were essentially the same in design. Women with a hrHPV-positive self-sample were advised to visit a general practitioner for a cervical smear and referred for colposcopy in case of abnormal cytology (threshold borderline or mild dyskaryosis (BMD), equalling AGC/ASC-US/ASC-H/LSIL). Those with normal cytology received a re-invitation for a cervical scrape after one year, and were referred for colposcopy if either hrHPV test result was positive or cytology was abnormal. Women of the recall control groups were managed according to the current cytology guidelines of the national screening programme.(15) For the purpose of this study data from these PROHTECT studies were pooled.

Screening participants

The pooled 18 month yields of ≥CIN2/≥CIN3 in the HPV sampling group were compared with those of all women (n=176,027) who did participate in the regular screening programme in the same region and the same period. These women were managed according to the current cytology screening guidelines(15).

Cytology and histology results of both the HPV sampling group and the screening participants were obtained by querying the nationwide, centralized network and registry of histology and cytology database (PALGA; Bunnik, the Netherlands(16)) as well as record tracking of individual cases of invited non-attendees. We linked patient records based on identity of the encrypted first four letters of the maiden name and date of birth. Groups of records presumably belonging to a single person were 'eyeballed' (checking every case manually) to filter out administrative twins by checking domicile, initials, and apparent inconsistencies in clinical history.

Study parameters

Response rate in PROHTECT was operationally defined as the proportion of eligible women of both arms who sent in an informed consent form, combined with submission of a self-sampled specimen for women assigned to the self-sampling group.(6;7) hrHPV prevalence was defined as percentage of women with HC2 hrHPV-positive self-sampled specimens.(6;7)

Yields of ≥CIN2/≥CIN3/cervical carcinoma refer to the 18-month cumulative yields of these lesions in women in the self-sampling group who submitted a self-collected specimen or women who participated in the screening programme.

Ethnic status of non-attendees defined by country of birth was retrieved from the invitational database of the Regional Health Council. In accordance with the method of the Dutch Central Bureau of Statistics, countries of origin (in total, n=188) were grouped into three major groups: The Netherlands (native Dutch), *Other Developed* countries (i.e., Europe, USA/Canada, Australia, New-Zealand) and *Developing* countries (i.e., the

major 4 immigrant populations in the Netherlands (The Netherlands Antilles, Surinam, Turkey, Morocco) and *Other Developing* countries).

In the Netherlands, women are invited for screening every five years in the year in which they reach the age of 30, 35, 40 etc. till 60 years. Age categorization was based on the number of prior screening rounds for which women had been invited. As a consequence the following age categories were defined: 29-33 years, 34-38 years, 39-43 years, 44-48 years, 49-53 years, 54-58 years, and 59-63 years.

For cytology screening history of non-attendees the time period between the invitation for HPV self-sampling and the last smear taken prior to the PROHTECT test was considered. For this subgroup comparison, only women who had been invited in one or more previous screening rounds (i.e., women aged 34-63 years; n=43,979) were included since younger women had no screening history. Since the PALGA database was linked with the invitational database for call and recall not earlier than in 2006, smears made for the invitational screening programme and opportunistic/diagnostic smears were similarly assigned. Based on time since the last smear, women were categorized into one of three subgroups: 1. last smear taken $\leq$7 years before participating to HPV self-sampling, considered to represent women screened in the previous round, 2. last smear taken >7 years ago (i.e., underscreened women) or 3. no smear in the past (i.e., never screened women). It should be noticed that PALGA has been virtually complete only since 1990 onwards (www.palga.nl). This means that the screening history can be screened only till 1990, and "no screening history in the past" is defined as no screening history in the past approximately 15 years.

Table 1: Characteristics of self-sampling groups of individual PROHTECT studies

	PROHTECT-1	PROHTECT-2	OR (95% CI)
Year of non-attendance	2005	2006	
Year of recruitment for study	2006-2007	2007-2008	
Device	Delphi Screener	Viba Brush	
Number of eligible women	26,886	25,561	
Response rate to HPV self-sampling	7,404 (27%)	7,870 (31%)	0·8 (0·8 - 0·9)
hrHPV positivity amongst self-sampling responders	757 (10·2%)	652 (8·3%)	1·3 (1·2 - 1·5)

Data analysis

The pooled 18-month cumulative ≥CIN2/≥CIN3 yields in self-sampling responders were compared with those of screening responders using Mantel-Haenszel (M-H) Chi-square testing. For analyzing the age stratified data we used the M-H test of homogeneity.

We performed multiple logistic regression analyses models on the potential risk factors as ethnic background, age group, and screening history. Outcome measures were response to HPV self-sampling invitation, hrHPV test result, ≥CIN2/≥CIN3/carcinoma. In the analyses for response to self-sampling invitation, the method of invitation (self-sampling or second recall) was also included as a predictor. Significance of the effects was evaluated with the Wald test. For all tests a significance level (α) of 0·05 was used.
The analyses were performed by using SPSS 15·0 software and STATA 10·0 package).

Results

HPV self-sampling responders of non attendees of the regular screening programme

In the PROHTECT studies, a total of 54,482 non-attendees were recruited, of whom 53,937 women were allocated to the self-sampling group and 545 to the recall control group. A total of 1,490 women were non-eligible, mainly due to previous hysterectomy, leaving 52,447 women in the self-sampling group. Seven women in the recall control group were non-eligible, leaving 538 women. Finally 15,274 women (29%) submitted a self-sampled specimen. Table 1 provides further details of the self-sampling groups of the individual PROHTECT studies.

Comparison of ≥CIN2/≥CIN3 yields between self-sampling responders and screening participants

Figure 1 and Table 2 show the pooled cumulative 18-month ≥CIN2/≥CIN3 yields in PROHTECT self-sampling responders versus screening participants. The ≥CIN2/≥CIN3 yields of self-sampling responders were higher than those of screening participants (≥CIN2: relative risk (RR)=1·6, 95%CI=1·4-1·9; ≥CIN3: RR=1·8, 95%CI=1·5-2·1). These relative risk values increased with age (Test of Homogeneity (M-H): ≥CIN2: p=0·04; ≥CIN3: p=0·03), but were also significantly higher than 1 in women aged 29–33 years (≥CIN2: RR=1·4, 95% CI=1·1-1·8; ≥CIN3: RR=1·6, 95% CI=1·2-2·2). When restricting the analysis to women who had abnormal cytology (≥BMD) at baseline similar relative risk values were obtained. In for example women aged 29–33 years with abnormal cytology these relative risks were 1·4 (95% CI=1·1-1·8) and 1·6 (95% CI=1·2-2·1) for ≥CIN2 and ≥CIN3, respectively.

Also cervical carcinomas were more frequently found among self-sampling responders than regular screening participants (0·09% vs 0·03%, p=0·002; Table 2). Due to the low number of carcinomas the effect of age could not be tested.

Figure 1: Yield of ≥cervical intraepithelial neoplasia (CIN)2 in pooled PROHTECT-1 and PROHTECT-2 studies and amongst women participated the regular cervical screening programme.

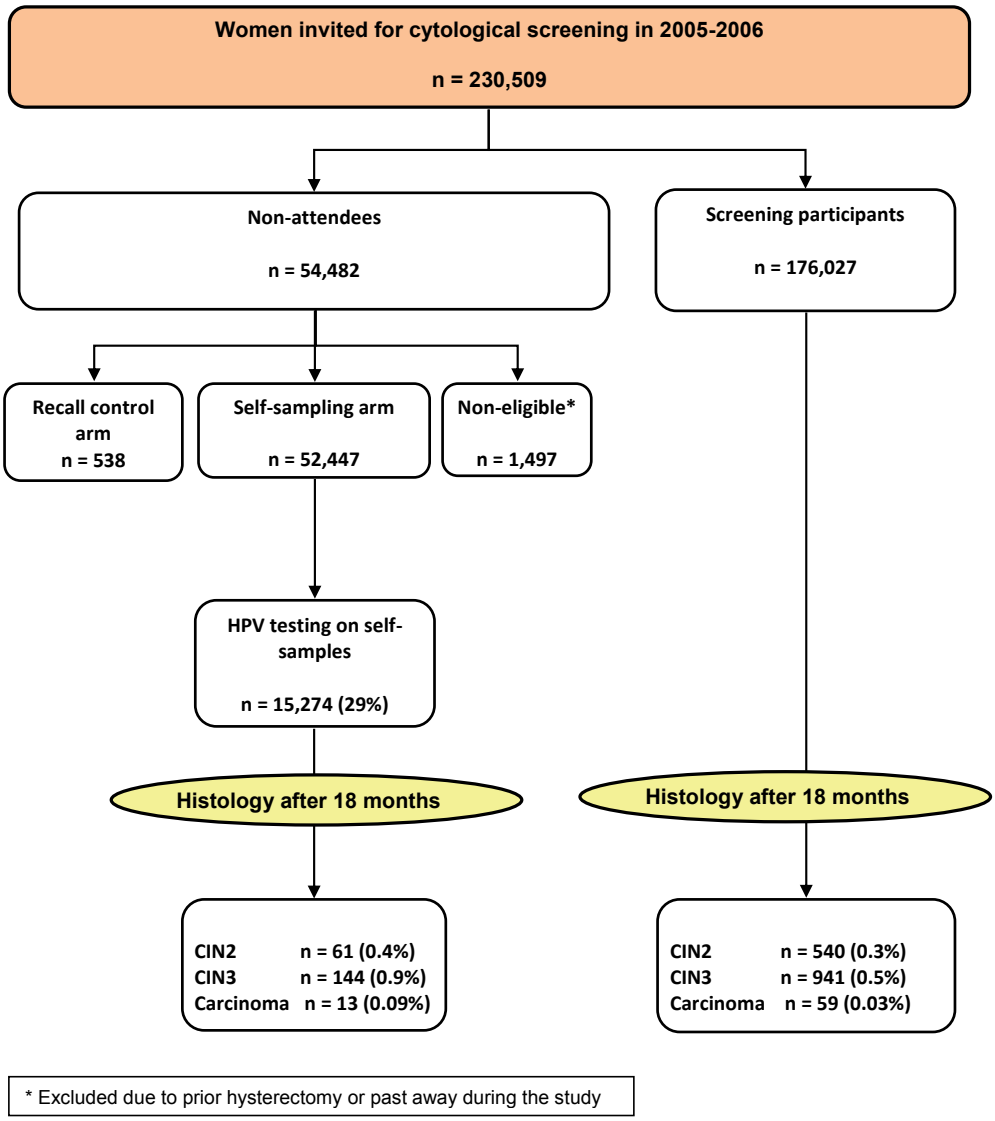


Table 2 – Response rate and yield of ⩾cervical intraepithelial neoplasia (CIN)2/⩾CIN3 and carcinoma in self-sampling responders compared to regular screening participants from the same region in 2005 and 2006.

Age	Responders PROHTECT								Participants regular screening programme (RSP)								PROHTECT versus RSP			
	Participants		⩾CIN2		⩾CIN3		Carcinoma		Participants		⩾CIN2		⩾CIN3		Carcinoma		⩾CIN2		⩾CIN3	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	Relative risk (RR)	95% Confidence interval	RR	95% CI
29–33 years	2328	27	71	3.1	54	2.3	2	0.09	21,524	61	463	2.2	308	1.4	4	0.02	1.418	(1.1, 1.8)	1.6	(1.2, 2.2)
34–38 years	3000	30	65	2.2	44	1.5	4	0.1	28,861	64	401	1.4	273	1.0	16	0.06	1.559	(1.2, 2.0)	1.6	(1.1, 2.1)
39–43 years	2612	30	26	1.0	17	0.7	1	0.04	27,315	71	277	1.0	172	0.6	19	0.07	0.982	(0.7, 1.5)	1.0	(0.6, 1.7)
44–48 years	2308	30	27	1.2	18	0.8	4	0.2	29,625	72	222	0.8	138	0.5	7	0.02	1.561	(1.0, 2.3)	1.7	(1.02, 2.7)
49–53 years	1904	30	12	0.6	10	0.5	1	0.05	25,827	72	102	0.4	63	0.2	6	0.02	1.596	(0.9, 2.9)	2.2	(1.1, 4.2)
54–58 years	1637	28	10	0.6	8	0.5	0	0.00	20,181	69	34	0.2	24	0.1	4	0.02	3.626	(1.8, 7.3)	4.1	(1.9, 9.1)
59–63 years	1485	27	7	0.5	6	0.4	1	0.07	22,694	59	41	0.2	22	0.1	3	0.01	2.609	(1.2, 5.8)	4.2	(1.7, 10.3)
Total	15,274	29	218	1.4	157	1.0	13	0.09	176,027	67	1540	0.9	1000	0.6	59	0.03	1.631	(1.4, 1.9)	1.8	(1.5, 2.2)

Table 3 – High-risk human papillomavirus (hrHPV) prevalence and ⩾CIN2/⩾CIN3/carcinoma yield in self-sampling group, and response rate in both self-sampling and recall control group, stratified by country of birth.

Country of birth	Response rate									Self-sampling group							
	Recall control group			Self-sampling group			Total			HPV		⩾CIN2		⩾CIN3		Carcinoma	
	Participants	Invited	Response (%)	Participants	Invited	Response (%)	Participants	Invited	Response (%)	*n*	%	*n*	%	*n*	%	*n*	%
The Netherlands	45	382	11.8	11,705	36,072	32	11,750	36,454	32	1062	9.1	184	1.6	131	1.1	10	0.09
Other developed countries	5	40	12.5	969	4036	24	974	4076	24	97	10.0	6	0.6	3	0.3	1	0.10
Developing countries[a] (subtotal)	13	116	11.2	2600	12,339	21	2613	12,455	21	250	9.6	28	1.1	23	0.9	2	0.08
Surinam	2	25	8.0	574	2930	20	576	2955	20	60	10.5	7	1.2	6	1.0	2	0.4
Netherlands Antilles	0	4	0.0	143	614	23	143	618	23	16	11.2	1	0.7	1	0.7	–	
Morocco	1	22	4.5	512	2451	21	513	2473	20	40	7.8	4	0.8	3	0.6	–	
Turkey	3	13	23	378	1613	23	381	1626	23	31	8.2	2	0.5	2	0.5	–	
Other developing countries	7	52	13.5	993	4731	21	1000	4783	21	103	10.4	14	1.4	11	1.1	–	
Total	63	538	11.7	15,274	52,447	29	15,337	52,985	29	1409	9.2	218	1.4	157	1.0	13	0.09

[a] The values in this row are the cumulative values summed over countries that are listed below in the shaded rows.

Response rate of non-attendees in relation to invitational method, ethnicity, age, and screening history

The response rate was analyzed by fitting a logistic regression model with method of invitation, ethnicity, age group, and screening history as predictors.

Women assigned to the self-sampling group responded significantly better than those assigned to the recall control group (29% versus 12%; $\chi^2(1)=73.9$, p<0·001, OR=3.2, 95% CI= 2.5-4.2; Table 2). The response rate was also related to ethnicity ($\chi^2(6)= 595.5$, p<0·001), age ($\chi^2(6)=26.6$, p<0·001), and screening history in women ≥34 years ($\chi^2(2)=429.4$, p<0·001).

Native Dutch women responded better than immigrant women ($\chi^2(1)=402·6$, p<0·001, OR=24, 95% CI=18-33), and of the immigrants, those from *Other Developed* countries revealed a higher response rate than those from *Developing* countries ($\chi^2(1)=8·6$, p<0·01, OR=2·0, 95% CI=1·3-3·1). No differences in response rate between subgroups of immigrants from *Developing* countries (i.e., Netherlands Antilles, Surinam, Turkey, and Morocco versus *Other Developing* countries) were found (Table 3).

There was no age trend in the response rate among PROHTECT-women (Table 4).

Amongst women of ≥34 years, those who were screened at the previous screening round revealed a higher response rate (7,259/21,185; 34%) than underscreened or never screened women (5,733/23,240; 25%; $\chi^2(1)=389·4$, p<0·001, OR=1·5, 95% CI=1·5-1·6). This difference was also evident when the analysis was restricted to women ≥39 years, who had been invited at least two prior screening rounds ($\chi^2(1)=420·9$, p<0·001, OR=2·7, 95% CI=2·5-3·0). Strikingly, amongst women ≥39 years, never screened women revealed a higher response rate (2,270/9,151; 25%) than underscreened women (2,039/9,024; 23%; $\chi^2(1)=33·3$, p<0·001, OR=1·2, 95% CI=1·2-1·3). This was evident for women of all ethnic groups, although for immigrant women from *Other Developing* countries this difference did not reach significance (Figure 2).

Table 4 – Response rate and high-risk human papillomavirus (hrHPV) prevalence in self-sampling responders stratified by age.

Age cohort	Total invited	Response rate	HPV-positive	95% Confidence interval
29–33 years	8468	2328 (28%)	364 (16%)	14.2–7.1%
34–38 years	9937	3000 (30%)	350 (11.7%)	10.5–12.8%
39–43 years	8576	2612 (31%)	211 (8.1%)	7.0–9.1%
44–48 years	7796	2308 (30%)	152 (6.6%)	5.6–7.6%
49–53 years	6435	1904 (30%)	132 (6.9%)	5.8–8.1%
54–58 years	5806	1637 (28%)	113 (6.9%)	5.7–8.1%
59–63 years	5429	1485 (27%)	87 (5.9%)	4.7–7.1%
Total (29–63 years)	5247	15,274 (29%)	1409 (9.3%)[a]	<0.001

[a] These percentages are based on the response rate as denominator.

HPV prevalence in relation to ethnicity, age, and screening history

Of the 15,274 women who submitted a self-sampled specimen, 1,409 (9·2%) were hrHPV-positive. Neither ethnicity ($\chi^2(6)=7·4$, p=0·3) nor screening history (women ≥34 years: $\chi^2(2)=0·2$, p=0·9) were found to be related to hrHPV prevalence. The proportion of hrHPV-positive women decreased with age till the age category 39-43 years (29–33 years: 15% ($\chi^2(1)=129·8$, p<0·001), 34–38 years: 11·7% ($\chi^2(1)=70·8$,

p<0·001), 39–43 years: 8·1% ($\chi^2(1)=7·0$, p<0·01, OR=1·3, 95% CI=1·1-1·5), and remained stable in older women (Table 4).

≥CIN2/≥CIN3 yield in relation to ethnicity, age, and screening history

Sixty one (0·4%) of the self-sampling responders had CIN2, 144 (0·9%) CIN3, and 13 (0·09%) cervical carcinoma (Figure 1). The overall ≥CIN2 and ≥CIN3 yields were 1·4% (n=218) and 1·0% (n=157), respectively (Table 3).

Both the ≥CIN2 and ≥CIN3 rates were related to ethnicity (≥CIN2: $\chi^2(2)=14·6$, p<0·001; ≥CIN3: χ^2 (2)=9·2, p<0·01). The ≥CIN2/≥CIN3 rates were higher among native Dutch women than among immigrants (≥CIN2: $\chi^2(1)=13·0$, p<0·01, OR=2·4, 95% CI=1·5-3·8; ≥CIN3: $\chi^2(1)=8·7$, p<0·01, OR=2·6, 95% CI=1·4-4·9). No significant difference was found between immigrant women from *Developed* countries and those from *Developing* countries. Due to the low frequencies of ≥CIN2 no further subdivision was made among women from *Developing* countries.

Table 5 – High-risk human papillomavirus (hrHPV) prevalence and yield of ⩾CIN2/⩾CIN3 and carcinoma in self-sampling responders of ⩾34 years in relation to screening history, stratified by ethnicity and age.

Country of birth	Last smear ⩽7 years					Last smear >7 years or never being screened before				
	Total	HPV-positive	⩾CIN2	⩾CIN3	CxCa	Total	HPV-pos	⩾CIN2	⩾CIN3	CxCa
The Netherlands										
34–38 years	1244	159 (12.8%)	27 (2.2%)	20 (1.6%)	1 (0.1%)	922	107 (11.6%)	29 (3.1%)	17 (1.8%)	1 (0.1%)
39–43 years	1192	101 (8.5%)	13 (1.1%)	9 (0.8%)	–	791	60 (7.6%)	8 (1.0%)	5 (0.6%)	1 (0.1%)
44–48 years	1087	67 (6.2%)	8 (0.7%)	7 (0.6%)	–	696	42 (6.0%)	16 (2.3%)	10 (1.4%)	3 (0.4%)
49–53 years	863	54 (6.3%)	5 (0.6%)	4 (0.5%)	–	615	37 (6.0%)	4 (0.7%)	4 (0.7%)	1 (0.2%)
54–58 years	786	48 (6.1%)	3 (0.4%)	1 (0.1%)	–	574	35 (6.1%)	5 (0.9%)	5 (0.9%)	–
59–63 years	739	40 (5.4%)	1 (5.4%)	–	–	547	34 (6.2%)	6 (1.1%)	6 (1.1%)	1 (0.2%)
Total	5911	469 (7.9%)	57 (1.0%)	41 (0.7%)	1 (0.02%)	4145	315 (7.6%)	68 (1.6%)	47 (1.1%)	7 (0.2%)
Other developed countries										
34–38 years	88	8 (9.1%)	1 (1.1%)	1 (1.1%))	–	115	18 (16%)	1 (0.9%)	1 (0.9%)	1 (0.9%)
39–43 years	73	7 (9.6%)	–	–	–	107	11 (10.3%)	1 (0.9%)	–	–
44–48 years	66	5 (7.6%)	–	–	–	84	5 (6.0%)	1 (1.2%)	–	–
49–53 years	59	5 (8.5%)	–	–	–	52	5 (9.6%)	1 (1.9%)	1 (1.9%)	–
54–58 years	27	1 (3.7%)	–	–	–	39	8 (21%)	–	–	–
59–63 years	35	–	–	–	–	37	4 (10.8%)	–	–	–
Total	348	26 (7.5%)	1 (0.3%)	1 (0.3%)	–	434	51 (11.8%)	4 (0.9%)	2 (0.5%)	1 (0.2%)
Developing countries[a]										
34–38 years	249	18 (7.2%)	3 (1.2%)	2 (0.8%)	–	382	40 (10.5%)	4 (1.0%)	3 (0.8%)	1 (0.3%)
39–43 years	203	13 (6.4%)	1 (0.5%)	1 (0.5%)	–	246	19 (7.7%)	3 (1.2%)	2 (0.8%)	–
44–48 years	198	15 (7.6%)	2 (1.0%)	1 (0.5%)	1 (0.5%)	177	18 (10.2%)	–	–	–
49–53 years	150	14 (9.3%)	–	–	–	165	17 (10.3%)	2 (1.2%)	1 (0.6%)	–
54–58 years	101	10 (9.9%)	–	–	–	110	11 (10.0%)	2 (1.8%)	2 (1.8%)	–
59–63 years	61	5 (8.2%)	–	–	–	66	4 (6.1%))	–	–	–
Total	962	75 (7.8%)	6 (0.6%)	4 (0.4%)	1 (0.1%)	1146	109 (16%)	11 (1.7%)	8 (1.2%)	1 (0.2%)
Overall										
34–38 years	1581	185	31	23	1	1419	165	34	21	3
39–43 years	1468	121	14	10	–	1144	90	12	7	1
44–48 years	1351	87	10	8	1	957	65	17	10	3
49–53 years	1072	73	5	4	–	832	59	7	6	1
54–58 years	914	59	3	1	–	723	54	7	7	–
59–63 years	835	45	1	–	–	650	42	6	6	1
Total (overall)	7221	570 (7.9%)	64 (0.9%)	46 (0.6%)	2 (0.03%)	5725	475 (8.3%)	83 (1.4%)	57 (1.0%)	9 (0.2%)

[a] Includes also Turkey, Morocco, Surinam and The Netherlands Antilles.

The ≥CIN2/ ≥CIN3 yields were significantly related to age (≥CIN2: $\chi^2(6)=52·3$, p<0·001; ≥CIN3: $\chi^2(6)=38·4$, p<0·001) and were relatively high in young women. Of all ≥CIN2 lesions, 32% were in the group of 29-33 years and only 3·2% were in the group of 59-63 years; likewise 34% of all ≥CIN3 were in the group of 29-33 years and 3·8% in women of 59-63 years.

The effect of screening history of women ≥34 years on ≥CIN2/≥CIN3/carcinoma yields, stratified by ethnicity and age, is shown in Table 5. There was a significant effect of screening history on both ≥CIN2 ($\chi^2(2)=11{\cdot}1$, $p<0{\cdot}01$) and ≥CIN3 ($\chi^2(2)=6{\cdot}6$, $p<0{\cdot}05$). Women who were under- or never screened revealed significantly higher ≥CIN2/≥CIN3 yields than women screened within the last 7 years (≥CIN2: χ^2 (1)=7·8, $p<0{\cdot}01$, OR=2·7, 95% CI=1·3-5·3) and ≥CIN3: $\chi^2(1)=4{\cdot}6$, $p<0{\cdot}05$, OR=2·5, 95% CI=1·1-5·5). A similar effect was seen after restricting the analyses to women ≥39 years (≥CIN2: $\chi^2(2)=14{\cdot}2$, $p<0{\cdot}001$); ≥CIN3: $\chi^2(2)=11{\cdot}4$, $p<0{\cdot}01$). The ≥CIN2/≥CIN3 yields were highest in never screened women (Figure 3).

Both in women of ≥34 and those of ≥39 years there was no significant effect of screening history on carcinoma yield. This reflects the fact that two carcinomas were diagnosed in women ≤33 years and the number of carcinomas in the older age groups was apparently too low to reach significance.

Discussion

In the screening region of the Netherlands investigated here the attendance rate was 67%, which is in agreement with the overall attendance in the Netherlands after one year. (65%)(17) Together with opportunistic smears the coverage of the population after 5 years is about 77%(17-19), which leaves 23% invited women unprotected. We showed that the yields of ≥CIN2/≥CIN3 were higher in the HPV self-sampling group of the non-attendees than in the regular attendees of the screening programme. Moreover, the relative ≥CIN2/≥CIN3 risk values increased with age.

In addition, we found that Dutch non-attendees responded better and also revealed significantly higher ≥CIN2/≥CIN3 yields than their immigrant counterparts. Amongst women invited at earlier screening rounds, never screened women responded better to HPV self-sampling than underscreened women, independent from ethnicity. These underscreened and never screened women displayed the highest risk of ≥CIN2/≥CIN3/carcinoma. These are the women who health programme managers particularly like to target to improve cervical cancer prevention strategies, supporting the notion that offering HPV self-sampling is a meaningful and effective approach for reaching those women who are in the highest need for cervical screening. Since non-attendees harbour more than 50% of cervical cancers(9;12), targeting of approximately 30% of these women by HPV self-sampling is likely to result in earlier detection of at least 15% of the cervical carcinomas.

For this study, we pooled data from two large self-sampling studies. Independent from ethnicity, age, and screening history, we measured different response rates between the individual PROHTECT studies. In PROHTECT-1 slightly fewer women responded than in PROHTECT-2 (27% vs 31%). This small difference may partly reflect a higher acceptability of the brush device used in PROHTECT-2 compared to the lavage-device used in PROHTECT-1. Alternatively, since PROHTECT-1 was performed prior to PROHTECT-2, the difference might be attributable to more awareness, and therefore less uncertainty, due to the earlier publicity around the PROHTECT-1 study.

Figure 2: Proportion self-sampling responders (aged ≥39 years) by screening history, stratified by ethnicity.

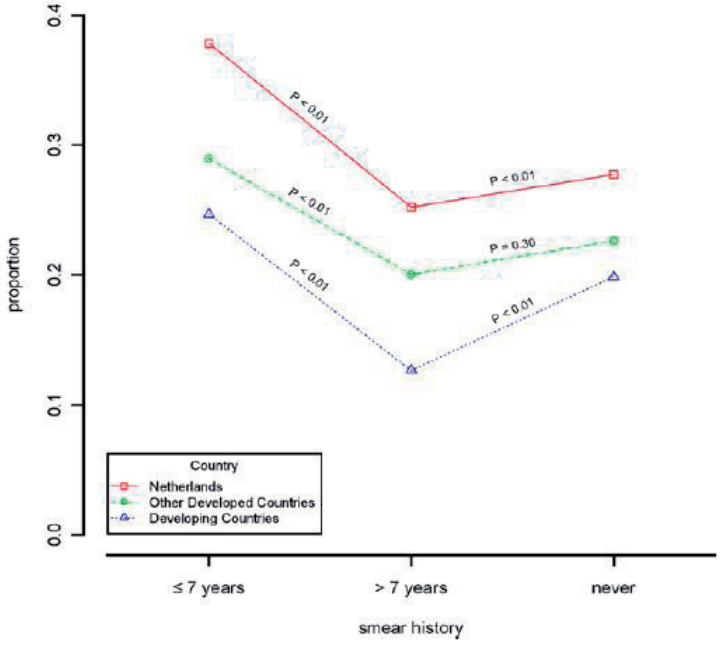
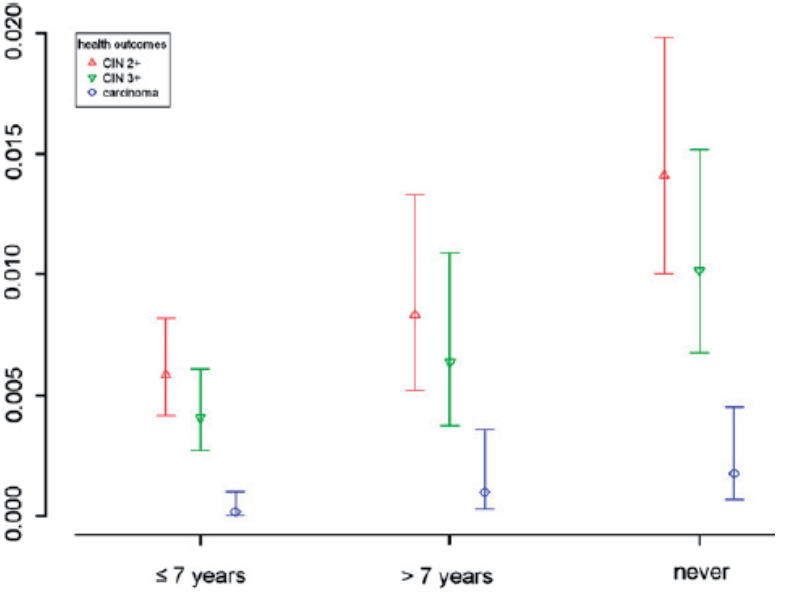


Figure 3:Yield of ≥cervical intraepithelial neoplasia (CIN)2/ ≥CIN3/carcinoma in self-sampling responders aged ≥39 years stratified by screening history. Lines represent 95% confidence intervals.



Most interesting is the finding that never screened women were more likely to respond than underscreened women, independent from the ethnic background. Although it is still unclear why never screened women responded better than underscreened women, a plausible explanation might be that these women consistently refuse to visit the physician for making a preventive smear because of cultural, religious and/or organisational reasons. HPV self-sampling may help to overcome this barrier.

It should be realized that increased ≥CIN2/≥CIN3 yield in self-sampling responders might be the result of a more sensitive screening test (hrHPV test used in self-sampling compared to the cytology test used for screening participants). However, similar relative risk values were obtained after restricting the analysis to women with abnormal cytology at baseline. Therefore, the increased relative risk of self-sampling responders cannot solely be attributed to a more sensitive screening test.

An unexpected observation was that an increased relative risk of ≥CIN2/≥CIN3 was also found among self-sampling responders for whom it was their first screening round. A likely explanation for this finding is that women at risk because of their lifestyle (e.g. in terms of sexual behaviour and smoking habits) are better targeted by offering HPV self-sampling than by invitation for a physician-collected cervical scrape. The increased relative risks by age most likely reflect an overall poorer screening history of older self-sampling responders.

Our study is unique, because of its large size and performance within the setting of the regular cervical screening programme. Moreover characteristics of non-attendees of the screening programme who responded to self-sampling for HPV testing has not been described before. A limitation is that we pooled two studies in which different collection devices were used. As reported earlier(7), hrHPV-positivity rates slightly differed between samples collected by both devices, but the concordance between hrHPV-positivity rates in both types of self-collected samples and corresponding physician-collected cervical samples was very high (over 90%) in women with ≥CIN2.(6;21) Furthermore, ≥CIN2 yield was comparable in both studies(7) indicating that it is unlikely that pooling the PROHTECT studies would influence the interpretation of the results.

Another limitation is that we did not test the prevalence of ≥CIN2 in women with hrHPV-negative self-sample test. The medical ethics committee considered follow-up of these women in light of the very high negative predictive value of the hrHPV test for ≥CIN2 an unnecessary burden.(22)

Finally we defined ethnic status based on the country of birth. Thus some women from ethnic minorities who were born in the Netherlands might have been classified as "native Dutch", even though culturally they may to some degree resemble paternal immigrant communities. Although this might play a role predominantly among younger women we think that the number of women concerned is limited. Most women who united with their husband by immigration in The Netherlands did so in the late late 1970's, begin 1980's. The number of women born from these immigrated women and invited for screening (30-60 years) constitutes in our opinion therefore a small minority

Finally, it is important to note that in order to make HPV self-sampling a successful alternative to physician-sampling, the whole organisation should be well controlled. This

involves the sequence of sending the invitation with the self-sampling kit, return sending by surface mail, hrHPV testing with a clinically validated test that is compatible with the self-sampling device, follow-up of hrHPV-positive women by triage cytology by a physician and follow-up of hrHPV-positive women with normal cytology after 6 months to 1 year. We showed earlier that compliance to direct cytology triage is high (≥90%) but that there is poor adherence to follow-up testing after 1 year (~60%), which needs careful attention.(6;7) Still, these results strongly argue to implement hrHPV testing on self-sampled material as an alternative for hrHPV testing on a physician taken scrape.

Conclusion

Amongst women who had not been screened in the previous screening round, those who were never screened before were preferentially attained when offering HPV self-sampling. This likely contributed to higher ≥CIN2/≥CIN3 yields than found in regular screening participants, which is highly relevant for the success of the screening programme. Although native Dutch women responded better than immigrants, the response rates among immigrants from different countries hardly differed, making the method successful independent from country of birth.

Contributors

CJLM Meijer was the project leader, designed the study with DAM Heideman, FJ van Kemenade, PJF Snijders, had full access to all data. M Gök and CJLM Meijer drafted the first version of the manuscript and ALM de Vries, FJ van Kemenade, DAM Heideman, J Berkhof, L Rozendaal, F Voorhorst, and PJF Snijders, commented on the next versions. M Gök, ALM de Vries and J Berkhof did the data analyses; J Berkhof was responsible for the data analyses. L Rozendaal and JAM Beliën were responsible for database management. DAM Heideman, PJF Snijders and CJLM Meijer were responsible for HPV DNA testing. M Babovic was responsible for the screening register of the Regional Screening Organisation Database. J Spruyt was responsible for communication with gynaecologists. FJ van Kemenade was responsible for coordination of cytological testing at the individual laboratories. All authors critically reviewed the manuscript and approved the final version.

Conflict of interest statement

CJLM Meijer has relationships with Qiagen (Gaithersburg, USA) and GSK; PJF Snijders has occasionally been advisory board member of Gen-Probe (San Diego, USA), Roche (Pleasanton, USA), and GSK (Rixensart, Belgium); DAM Heideman has occasionally been invited speaker by Roche (Pleasanton, USA). CJLM Meijer, PJF Snijders, and DAM Heideman are shareholders of Self-screen, a recent spin-off company of VU University medical center. The sources of funding did not have any influence on the design and the analysis of the results.

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Chapter 6

HPV16 and increased risk of recurrence after treatment for CIN

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Abstract

Objective Addition of high-risk human papillomavirus (hrHPV) testing to post-treatment monitoring policies of women treated for high-grade cervical intraepithelial neoplasia (CIN) may improve the effectiveness of detecting recurrent/residual disease. Recent studies have shown that HPV type 16 confers an increased risk of high-grade CIN and cervical cancer. This study aimed to find out whether the post-treatment CIN 3 rate is increased in HPV 16 positive women treated for CIN 3.

Methods We included 229 hrHPV positive women treated for CIN 3. HPV typing was performed by GP5+/6+-PCR followed by reverse line blotting on a cervical scrape taken before treatment. HPV typing data were related to the occurrence of post-treatment CIN 3 within a median follow-up time of 20.1 months (range 3-85.4 months) following treatment.

Results Twenty nine of the 151 (19%) HPV 16 positive women versus 6 of the 78 (8%) women with other hrHPV types had recurrent/residual CIN 3. Post-treatment CIN 3 rate was significantly increased in women with HPV 16 compared to those harbouring other hrHPV types (p=0.03). None of the other hrHPV types were associated with higher post-treatment CIN 3 rates.

Conclusion Women treated for HPV 16 containing CIN 3 should be monitored more intensively because of their increased risk of post-treatment CIN 3. Thus, the HPV genotype should be considered in post-treatment monitoring policies.

Introduction

Women treated for high-grade cervical intraepithelial neoplasia (CIN 2/3) are traditionally followed for at least 2 years by cytology to check for recurrent or residual disease. Post-treatment CIN rates of 5-15% have been observed [1]. For the development, maintenance, and progression of CIN 2/3 lesions, a persistent infection with high-risk human papillomavirus (hrHPV) is required [2,3]. Moreover, radical removal of CIN 2/3 lesions is associated with negative HPV test results in cervical smears [4]. Consequently, addition of hrHPV testing to post-treatment monitoring policies improves the effectiveness of detecting recurrent/residual disease [5-9].

Recent data indicate that among the hrHPV types, HPV16 confers by far the highest risk of high-grade CIN lesions and cervical cancer [10-12]. This raises the question whether there also exists an increased risk of post-treatment CIN in case of HPV16 infections compared to infections with other hrHPV types. In this study, we tested the hypothesis that an HPV16 infection in a primary CIN3 lesion increases the risk of a recurrent/residual CIN3 after treatment.

Patients and methods

We used clinical follow-up and hrHPV typing data collected during the course of two studies [6,13], involving in total 332 women that were treated for CIN2/3. For the purpose of this study these data were pooled since the same method of hrHPV testing was performed within a single laboratory under guidance by the same investigators (departments of Gynaecology and Pathology, VU University Medical Center, Amsterdam). The mean age at baseline (time of treatment) was 35 years (range 21-64 years). The median follow-up time was 20.1 months (range: 3.0-85.4 months).

Standard follow-up in The Netherlands consists of repeat smears with cytological examination at 6, 12 and 24 months. Cervical smears were read according to the CISOE-A classification, and interpreted as normal, borderline dyskaryosis (BMD), mild dyskaryosis, moderate dyskaryosis, severe dyskaryosis, suspected of carcinoma in situ, or suspected of carcinoma. Translation of this classification system into the Bethesda 2001 classification has recently been described [14].

For the purpose of the abovementioned studies, extra repeat smears were taken at 3 and 18 months and, in addition to cytology, hrHPV testing was performed for 14 hrHPV types (i.e., HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58,59,66 and 68) by HPV consensus GP 5+/6+-PCR EIA followed by typing using reverse line blot analysis of PCR products [15].

According to guidelines currently in practice in The Netherlands, colposcopic examination including sampling for histological verification of suspect lesions was performed in case of a cytology reading of mild dyskaryosis or worse. Follow-up ended after treatment for recurrent/residual disease. Time to recurrence/residue was measured in days.

Statistical analyses

We selected women treated for CIN3 and compared the post-treatment CIN3 rate for those with HPV16 at baseline to that of women with other hrHPV types at baseline. In addition, the post-treatment CIN3 rate of women with HPV 18 was compared to those harboring hrHPV types different from both HPV16 and 18. HPV16 infections combined with other hrHPV types were considered as HPV16 multiple infections. Estimates of post-treatment CIN3 rates were based on Kaplan-Meier survival analyses to correct for loss to follow-up. Testing for differences between the Kaplan-Meier curves was done with the log rank test. Crude estimates of the post-treatment CIN3 rates are given as well. We carried out the analyses both for single infections and multiple infections combined and for single infections only.

Table 1: Type-specific post-treatment CIN 3 rates for women with a primary hrHPV positive CIN 3 lesion. Both women with multiple and single infections combined and women with single infections solely are shown.

hrHPV type	Total N	Crude estimates of the recurrence rate (%)	Adjusted estimates of the recurrence rate (%)†	p-value‡
Multiple and single infections				
16 vs other hrHPV	151 vs 78	19 vs 8	25 vs 8	0.03
18 vs other hrHPV*	17 vs 61	0 vs 10	0 vs 10	0.19
Single infections				
16 vs other hrHPV	135 vs 64	20 vs 8	26 vs 8	0.04
18 vs other hrHPV*	12 vs 52	0 vs 10	0 vs 10	0.29

* HPV 16 is excluded from this comparison

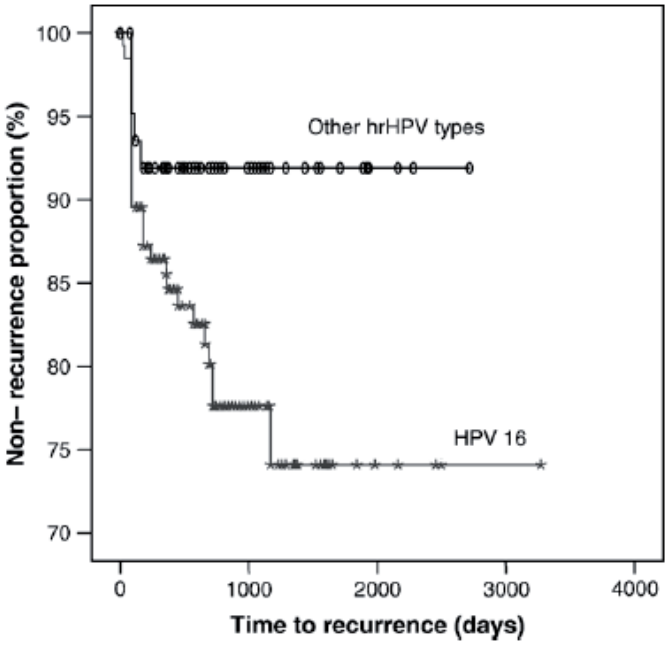
† Adjusted for loss to follow-up with Kaplan-Meier analysis

‡ Based on the log rank test

Of the 332 women, 229 had a hrHPV-positive CIN3 lesion at baseline (time of treatment). The remaining 103 women either had a hrHPV-positive CIN 2 lesion at baseline (83 women) or were hrHPV negative (20 women). Table 1 shows the type-specific post-treatment CIN3 rates, both for women with multiple and single hrHPV infections combined, and women with a single infection solely. Of the 229 hrHPV-positive women with CIN3, 151 (66%) contained HPV16, of which 16 (11%) were infected with one or more other hrHPV types as well. The latter included 3 women with HPV16/18 double infections. Another 17 women had HPV 18, 5 (29%) of which involved multiple infections with types different from HPV16. The remaining 61 women were infected with hrHPV types other than HPV16 and 18. In this group 9 (15%) women had a multiple infection.

Crude post-treatment CIN3 rates were 19% (29 of 151) and 8% (6 of 78) for women with HPV16 single and multiple infections combined and other hrHPV types, respectively. These figures did not change markedly when considering only multiple infections with and without HPV16. Crude post-treatment CIN3 rates were 13% (2 of 16) versus 7% (1 of 14) for women with multiple infections containing HPV16 versus those having multiple infections with types different from HPV16. Post-treatment CIN3 rates adjusted for lost to follow-up were 25% (HPV16 single and multiple infections combined) and 8% (other hrHPV types). These differences were statistically significant ($p = 0.03$). None of the 20 women in which no hrHPV was detectable revealed post-treatment CIN3.

Figure 1: Cumulative recurrence of CIN 3 after treatment for hrHPV positive CIN3 for women with HPV 16 versus other hrHPV types at baseline. Only single infections are included.



When taking into account women with primary CIN 2 and CIN3 lesions and focusing on the combined post-treatment CIN 2 and CIN3 rates similar results were obtained, i.e., a significant difference in post-treatment CIN 2/3 rate between women with HPV16 compared to those with other hrHPV types when single and multiple infections were considered together (data not shown).

After excluding the 151 women with HPV16, no post-treatment CIN3 was observed in the 17 women with HPV 18 at baseline (crude estimate 0%, corrected estimate 0%), whereas 6 of the remaining 61 women displayed CIN3 after treatment (crude estimate 10%, corrected estimate 10%). This difference, however, was not statistically significant ($p = 0.19$).

The corresponding Kaplan-Meier curves for women with single HPV16 infections versus those with single infections with other hrHPV types are shown in Fig. 1. Twenty seven of the 135 HPV16 single infected women (20%) versus 5 of the 64 (8%) women having a single infection with a different type presented with a post-treatment CIN3. Single infections with HPV16 infections revealed a significantly increased adjusted post-treatment CIN3 rate (26% versus 8%; $p = 0.04$).

Discussion

We have shown that the post-treatment CIN3 rate in women containing HPV16 at the time of treatment is significantly higher than in women with other hrHPV types. Our results are in line with recent findings that HPV16 exhibits a lower clearance rate than other hrHPV types [16,17] and an increased CIN 2/3 risk [10-12,18]. Moreover, HPV16 accounts for the majority of hrHPV infections in HPV containing carcinomas of sites different from the uterine cervix [19]. All these findings suggest that HPV16 is more oncogenic than other hrHPV types.

Previous studies have addressed the value of hrHPV testing in addition to cytology in detecting recurrent or persistent lesions after treatment for primary CIN (reviewed by Zielinski et al. [20] and Arbyn et al. [5]). The results of our study indicate that there is also a potential value for hrHPV genotyping. Despite the relatively small numbers of patients in this retrospective study, our data suggest that women with a primary lesion containing HPV16 should be monitored with more intensive awareness after treatment, given their increased risk of recurrent/residual disease. In this context a possible algorithm could be a 6-month follow-up of HPV16-positive women not only by cytology, as is currently the standard, but also by hrHPV typing. In case the follow-up smear is HPV16-positive a colposcopical examination should be advised, irrespective of the cytological outcome. Larger prospective studies are warranted to investigate the efficacy of such an alternative post-treatment management of women treated for HPV16 containing high-grade CIN.

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Chapter 7

Summary & Discussion

Literature data indicate that a substantial number of cervical carcinomas diagnosed in countries with a running, organised cervical screening programme is found in women who have not responded to an invitation for cervical screening.(1;2) Targeting these non-attending women is therefore of utmost importance to reduce the incidence of and mortality from this disease.

In this thesis we firstly evaluated the screening history of women with cervical cancer in the Netherlands in order to get a better insight into this problem in relation to age and FIGO stage. In addition, this thesis reports on the results of implementing hrHPV testing on self-sampled (cervico)-vaginal material from non-attendees of the cervical screening programme. Response rates, hrHPV prevalence, compliance to follow-up, referral to physician and treatment, and yield of high-grade CIN and cervical carcinoma were investigated in relation to age, ethnicity and screening history.

In **Chapter 2**, the adherence to the screening programme of women with cervical carcinoma was analysed in relation, age and FIGO stage of cervical cancer. We found that little more than half of the women with histologically-confirmed cervical carcinoma who were eligible for invitation for the screening programme were not screened in the last screening round, thus demonstrating that women with cervical cancer are still underscreened. Moreover, being underscreened or unscreened significantly correlated with higher cervical cancer stages, especially in older women. Advanced carcinoma or FIGO stage $\geq$2B occurred in 48.7% of poorly screened women as compared to 16.0% of screened women ($p<0.001$). Our results were in line with other studies in different settings and different populations.(1;3) Finally, in those women that were screened, we noted poor timeliness with respect to follow-up/referral strategies. Therefore, ways to increase adherence to screening and to optimise organisation aspects of the screening programme are important to improve the effectiveness of the screening programme.

In the Netherlands, several possibilities have been investigated to improve the attendance rate to screening, such as inviting women by their own GPs rather than health authorities(4;5), increased computerized support to practices and delegation of tasks to nurse practitioners.(6) However, the effect on attendance of these strategies is limited. A substantial proportion of women is still unwilling to visit their physician for making a cervical smear. Reasons for this include embarrassment, language and cultural difficulties with immigrant groups, time impediments for working women and improper individual risk assessment.(7)

Previous studies from our group have shown that offering a self-sampling device for collecting cervico-vaginal material for hrHPV testing may improve the participating rate.(8-12) A Dutch pilot study showed that by offering a self-sampling device, 34% of non-attendees are willing to participate to screening, compared to 17% of women who received a second reminder for regular screening.(9) Moreover, this pilot showed that the yield of high-grade CIN and cervical cancer (CIN2+) was higher in self-sampling responders compared to screening participants (1.67 vs. 0.97%), suggesting hrHPV

testing on self-sampled cervico-vaginal specimens to be an effective alternative to protect non-attendees in the cervical screening programme.

In **Chapters 3** and **4**, we continued the evaluation of offering self-sampling for hrHPV testing to non-responders of the regular cervical screening programme in a large, population-based screening setting (i.e. regions Noord-Holland and Flevoland of the Netherlands). The studies were entitled PROHTECT (*PRotection by Offering HPV TEsting on Cervico-vaginal specimens Trial*) 1 and 2. The first study evaluated a lavage device as self-sampling method (Figure 1a as used in PROHTECT-1 study, and Figure 1b shows an updated version), and in the second study a brush was offered (Figure 2). Main outcome measures were the response rate in comparison with sending a screening reminder invitation and yield of CIN2+/CIN3+. In addition, the concordance of HPV-test results between material sampled by the general practitioner and self-sampled cervico-vaginal material was determined.

Both studies showed that response rate in the self-sampling group was significantly higher than in the control group that received a recall for regular cytology screening (PROHTECT-1: 27.5%, and PROHTECT-2: 30.8%; $p<0.001$ in both studies). The yield of CIN2+ and CIN3+ lesions in self-sampling responders did not differ between both studies (i.e. 1.3% and 1.0%, respectively, in the first study, and 1.5% and 1.0%, respectively, in the second study. The overall concordance of HPV-test results between the GP-taken smear and the self-sampled specimen was 58.7% and 68.8% in PROHTECT-1 and PROHTECT-2, respectively. However, in women with CIN2+ and CIN3+, very high concordance figures were obtained (93.8% and 95.5%, respectively). Together, these data indicate that offering self-sampling for hrHPV testing is a much better alternative for women not attending the screening programme than sending them a second screening reminder letter.

In **Chapter 5** we present the overall results of pooled analyses of both PROHTECT studies with special emphasis on ethnicity, age, and screening history. Although the self-sampling response rate was higher in native Dutch women than in immigrants (32.4% versus 21.8%, $p<0.01$) no marked differences were found between immigrants born in non-developed countries (21.1%) compared to those of developed countries (24.0%). Thus, the lower response rate amongst immigrants can not simply be attributed to differences in ethnicity. Moreover, the response rate was independent of age. In self-sampling responders, who had not participated in the previous round of screening, increased rates of CIN2+/CIN3+/cervical carcinoma were found compared to women who had been screened in the last invitation round.(1.4%, 1.0%, 0.2% versus 0.9%, 0.6%, 0.03%, respectively). These rates were even higher in never screened women and were independent from ethnicity. Thus screening history appeared the main determinant for risk of high-grade CIN and cervical cancer. These results indicate that offering self-sampling is a feasible and effective method to protect non-attendees of cervical screening, irrespective of their ethnic background, and that the highest benefit of this approach can be expected for underscreened and unscreened women.

Overall, data from this thesis revealed that offering self-sampling for HPV testing to non-attendees in cervical screening, would increase the coverage by at least 5.2%. The yield of CIN2+/CIN3+ and carcinoma, found in both PROHTECT cohort studies, was significantly higher than the yield of CIN2+/CIN3+ and carcinoma among women with a smear in the last invitation round. Therefore, we can conclude that the results of the PROHTECT studies strongly support the implementation of this method for women not attending the regular screening programme.

The effect of the screening programme is, however, also dependent on the follow-up strategies, for instance for women diagnosed with high-grade CIN. Recent studies have shown that HPV type 16 confers an increased risk of high-grade CIN and cervical cancer.(13-15) Therefore, addition of hrHPV testing and genotyping to post-treatment monitoring policies of women treated for high-grade CIN may improve the effectiveness of detecting recurrent/residual disease. In **Chapter 6**, we studied whether the post-treatment CIN3 rate is increased in HPV16-positive women treated for CIN3. HPV typing was performed on a cervical scrape taken before treatment using the GP5+/6+-PCR method followed by reverse line blot assay. The results showed that post-treatment CIN3 rate was significantly increased in women with HPV16 compared to those harbouring other hrHPV types (p=0.03). None of the other hrHPV types were associated with higher post-treatment CIN3 rates. For this reason, we advise that women treated for CIN3 lesions, with HPV16 as underlying cause, should be monitored more intensively because of their increased risk of post-treatment CIN3.

Discussion and future perspectives

For years cervical scrapes taken by health professionals did constitute the basis for preparing conventional smears or liquid based cytology samples for cervical cancer screening. During the last years several efforts have been made to evaluate whether self-collected (cervico-)vaginal material could serve as a good alternative for physician-collected cervical scrapes. Dacron- or cotton swabs, brushes and tampons or various lavage devices have been used as collection devices. Data from others and us have shown that offering self-sampling can improve screening attendance in developed countries (9;16;17)and facilitate access to cervical screening in developing regions possible.(18;19) In addition, interview surveys in which participants were asked for collection preference have shown that women prefer self- collection over physician-collection.(20-23) Time and place of sampling, privacy and ease of sampling have been mentioned as advantages of self-sampling. Thus, there is a basis for self-sampling of vaginal- or cervico-vaginal specimens in cervical cancer screening.

However, self-collected vaginal samples are not suited for accurate cytological assessment, because of lower specimen quality (low cellularity)(24) poor concordance with cytology on conventional smears taken by a physician(25;26) and much lower sensitivities for high-grade cervical disease.

Many studies have therefore focused on the use of self-samplers for HPV analysis.(22;27) Collectively, these studies have shown that self-sampling can be as efficient as physician-sampling in detecting hrHPV. Discordance in hrHPV detection rates between self- versus physician-collected samples, as has been reported in some studies, most likely reflect the use of different types of self-sampling devices (swap, brush, tampon or lavage) that will influence the cell yield, as well as different hrHPV detection methods that all have their specific features in terms of analytical sensitivity and specificity for hrHPV detection. Most importantly, however, is knowledge about the performance of hrHPV self-sampling with regard to disease outcome.

Studies that have compared hrHPV testing on self-samples with cytology on physician-obtained cervical samples have shown that hrHPV testing on self-samplers is as sensitive or more sensitive for CIN2+ than cytology on physician-obtained cervical samplers.(25;28-33) Thus, hrHPV testing on self-collected samples is a safe alternative for cytology testing on physician-taken samples.

Despite some variations between studies, also sufficient evidence has been collected that highly concordant results can be obtained between hrHPV testing on self- and physician-sampled specimens at the level of CIN2+ detection.(18;23;25;29) This is supported by data from the non-attendees of our PROHTECT-1 study.(17) Relative to their (hrHPV plus cytology triage) screened counterparts of the same age category in the regular screening programme, non-responder women of $\leq$ 33 years demonstrated a similar CIN2+ rate (i.e. 0.8%; RR 0.81; 95% CI 0.53 – 1.21). Since women of this age had no previous screening round, this strongly suggests that the sensitivity for CIN2+ of hrHPV testing on self-samples is not inferior to that of hrHPV testing on physician-collected cervical samples. Variations in reported study results likely reflect the use of different collection devices and HPV tests and protocols Therefore, for reaching clinical equivalence in terms of detecting CIN2+ the right combination of self-sampler and validated hrHPV test is likely to be important.

Given abovementioned properties, it can be envisioned that hrHPV-testing on self-sampled specimens may have value as an alternative screening tool in regular, population-based screening and/or monitoring of women treated for CIN2+. Therefore, the time has come for an implementation study in which HPV testing on self-sampled cervico-vaginal material is offered as an alternative for HPV testing on a physician taken smear for CIN2+ detection in the regular screening programme. Also the Dutch Health Council has recently advised to perform such a study.

In addition, substantial improvements in the context of triage testing can be foreseen. Since cytomorphology on self-sampled specimens is not an option, women who tested hrHPV-positive on their self-sample are currently advised to visit a physician for a cervical smear. Application of molecular triage testing (i.e., testing for the presence of CIN2+ disease-related markers by molecular analysis) directly on the self-sampled specimens is nowadays a feasible option. A recent study presents an objective methylation marker panel (i.e., CADM1 and MAL) that was equally discriminatory for CIN3+ as cytology or cytology with HPV16/18 genotyping on physician-taken cervical smears in hrHPV-positive women.(34) The efficacy of this molecular triage strategy on

self-samples is currently being evaluated against that of triage via the general practitioner in the PROHTECT-3 trial. Molecular triage on self-samples opens the possibility for complete women-friendly cervical screening using objective, non-morphological molecular methods.

Figure 1a: the self-sampling lavage device (Delphi®-Screener), used in PROHTECT-1

Figure 1b: Updated version of the Delphi® screener presently in use

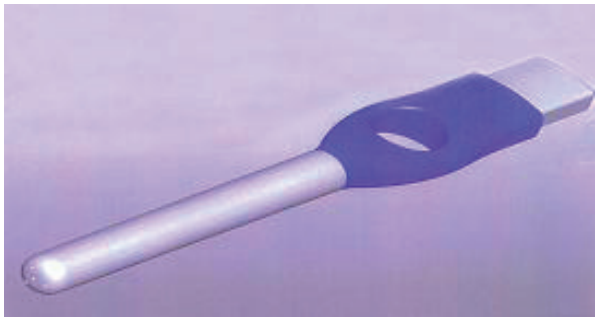
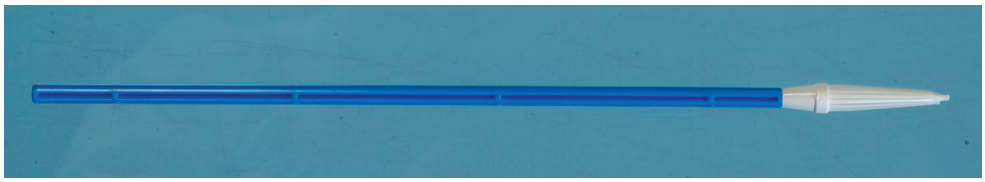


Figure 2: the self-sampling brush device (Viba-Brush®)



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Chapter 8

Nederlandse samenvatting

(HPV testen op zelf verzameld materiaal afkomstig van de cervix/vagina: een nieuwe manier van vrouwvriendelijke screenen op baarmoederhalskanker)

8.1 Baarmoederhalskanker en voorloperstadia

8.2 Humaan papillomavirus (HPV)

8.3 Het bevolkingsonderzoek naar baarmoederhalskanker (BVO BMHK)

8.4 Niet-deelneemsters binnen het BVO BMHK

8.5 De thuistest als oplossing voor niet-deelneemsters

8.6 Conclusie

List of publications

Acknowledgement

Curriculum Vitae

8.1 Baarmoederhalskanker en voorloperstadia

Baarmoederhalskanker is wereldwijd één van de meest voorkomende vormen van kanker bij vrouwen. Baarmoederhalskanker komt voor bij vrouwen van alle leeftijden, maar het meest bij vrouwen in de leeftijd van 35 tot 50 jaar. Elk jaar krijgen in Nederland ongeveer 630 vrouwen baarmoederhalskanker, en jaarlijks overlijden ongeveer 230 vrouwen aan deze ziekte. Een infectie met het humaan papillomavirus (HPV) is noodzakelijk voor het ontstaan van baarmoederhalskanker.

Baarmoederhalskanker kan ontstaan wanneer het evenwicht tussen de opbouw en de afbraak van het slijmvlies van de baarmoedermond verstoord is. Meestal worden deze afwijkingen veroorzaakt door een ontsteking of infectie, bijvoorbeeld met HPV. Bij zo'n ontregeling kunnen afwijkingen van de baarmoedermond, zogenaamde laesies, ontstaan. Deze afwijkingen verdwijnen vaak vanzelf door optreden van het afweersysteem (immuunsysteem) van de vrouw. Mocht het immuunsysteem de oorzaak van deze laesies zoals de HPV infectie niet kunnen onderdrukken, dan kunnen steeds meer afwijkende cellen ontstaan. Men spreekt dan van een voorstadium van baarmoederhalskanker. Deze voorstadia worden CIN (= cervicale intraepitheliale neoplasie) graad 1 (CIN1), - graad 2 (CIN2) en - graad 3 (CIN3) genoemd. Zonder behandeling kunnen deze CIN laesies zich uiteindelijk ontwikkelen tot baarmoederhalskanker. Het proces van het ontwikkelen van een voorstadium tot baarmoederhalskanker verloopt heel langzaam en kan wel tien tot vijftien jaar duren.

8.2 Humaan papillomavirus (HPV)

Baarmoederhalskanker wordt veroorzaakt door een voortdurende (persistente) infectie met een kankerverwekkend, zogenaamd hoog-risico, type van HPV. Deze ontdekking heeft geresulteerd in nieuwe mogelijkheden voor preventie van baarmoederhalskanker, namelijk profylactische HPV vaccins en het gebruik van de HPV test (d.w.z. een test die de aan- of afwezigheid van het virus meet) binnen het bevolkingsonderzoek naar baarmoederhalskanker (BVO BMHK).

HPV infecties komen veel voor en er zijn meer dan honderd verschillende types van het virus bekend. Van een deel van deze types, ongeveer 15, is bekend dat zij baarmoederhalskanker kunnen veroorzaken. Deze type virussen worden dan ook oncogene (kankerverwekkende) typen, of hoog risico HPV (hrHPV) typen genoemd.

HPV infectie van de baarmoedermond kan ontstaan door geslachtsgemeenschap. Een infectie van de baarmoedermond met HPV komt gedurende het leven voor bij 80% van alle vrouwen. Bij de meerderheid, ~90%, van de geïnfecteerde vrouwen is het lichaam in staat het virus te klaren met haar eigen immuunsysteem. Wanneer het immuunsysteem niet in staat is de virusinfectie op te ruimen, blijft het virus in het slijmvlies van de baarmoedermond zitten en kunnen de bovengenoemde voorstadia (CIN laesies) onstaan. Bij de lichte vorm van deze

voorstadia zoals CIN1 kan de infectie, en daarmee de laesie, nog in 95% van de gevallen genezen. Bij CIN2 en CIN3 wordt de kans op volledig herstel steeds kleiner. Een klein deel van alle vrouwen waarin de infectie niet geneest, kan na verloop van 10 of meer jaar baarmoederhalskanker ontwikkelen hetgeen gepaard gaat met veranderingen in het genoom (ofwel het kernmateriaal) van de geïnfecteerde cel. Deze veranderingen zorgen voor de functionele veranderingen van de hrHPV-geïnfecteerde cel, waardoor deze ongecontroleerd kan gaan groeien.

8.3 Het bevolkingsonderzoek naar baarmoederhalskanker (BVO BMHK)

De lange periode van voorstadia bij het ontstaan van baarmoederhalskanker biedt de mogelijkheid voor het vroeg detecteren van deze ziekte. Vroege detectie maakt betere behandeling mogelijk en verhoogt de kans op genezing, en kan daarmee het ontstaan van baarmoederhalskanker voorkomen. Het bevolkingsonderzoek naar baarmoederhalskanker (BVO BMHK) is al in de jaren ’70 van de vorige eeuw geïntroduceerd, met als doel het opsporen van voorloopstadia en het aantal gevallen van baarmoederhalskanker te reduceren. In 1989 was er min of meer landelijke dekking van het BVO BMHK. Niettemin, in 1996 was grondige herziening van de organisatie van het BVO BMHK nodig, om meer vrouwen deel te laten nemen en om het aantal opportunistische uitstrijkjes terug te dringen. Ook het aantal herhalingsuitstrijkjes werd teruggedrongen door nieuwe classificatie (beoordeling van de uitstrijkjes) in te voeren. Sindsdien worden in Nederland vrouwen tussen de 30 en 60 jaar om de 5 jaar voor het BVO BMHK opgeroepen om een uitstrijkje te laten maken bij de huisarts. Bij het detecteren van abnormale cellen in dit uitstrijkje wordt de patiënte doorverwezen naar de gynaecoloog. De periode sinds 1996 tot heden is eigenlijk nog te kort om daadwerkelijk effecten te kunnen meten van de invoor van de veranderingen. Toch is er sinds 1989 wel een vermindering van de incidentie van baarmoederhalskanker opgetreden van 9,1 per 100.000 vrouwen in 1989 tot 7,9 per 100.000 vrouwen in 2007. Dit is een vermindering van circa 13%.

8.4 Niet-deelneemsters binnen het BVO BMHK

Ondanks de landelijk invoering van BVO BMHK in 1989 is de dekkingsgraad (= het percentage vrouwen met een baarmoeder/baarmoedermong dat een uitstrijkje laat maken) niet volledig. Slechts 77% van alle vrouwen hebben, gemeten over één ronde van het BVO BMHK, een uitstrijkje laten maken. Drie-en-twintig procent van de vrouwen laat (bijna) nooit in hun leven een uitstrijkje maken (zogenaamde niet-deelneemsters).

In ***hoofdstuk 2*** hebben we aangetoond dat 55% van de gevallen van baarmoederhalskanker gediagnosticeerd wordt onder niet-deelneemsters. Klaarblijkelijk hebben niet-deelneemster een hoger risico op het ontwikkelen van baarmoederkanker dan vrouwen die wel deelnemen aan het BVO BMHK. Omdat niet-deelneemsters zich niet laten controleren worden mogelijke voorloopstadia gemist, met als gevolg dat de voorloopstadia zich verder kunnen ontwikkelen tot baarmoederhalskanker.

Het is lastig te onderzoeken wat de motieven zijn van vrouwen om niet deel te nemen aan het BVO BMHK, maar verschillende onderzoekingen wijzen op een rol van de volgende factoren: geen vertrouwen in de huisarts/medische wereld, geen tijd (werkende vrouwen), vanwege religieuze redenen / cultuur, misvatting omtrent het risico op baarmoederhalskanker, en gevoelens van schaamte en/of angst voor de ingreep (pijn, ongemak) bij de huisarts.

8.5 De thuistest als oplossing voor niet-deelneemsters

Om de vrouwen die niet willen of kunnen deelnemen aan het BVO BMHK een alternatief te bieden, hebben we in 2006 het PROHTECT thuistestonderzoek opgezet. Dit onderzoek was bedoeld voor vrouwen die in 2005 of 2006 uitgenodigd waren voor het BVO BMHK maar niet deelgenomen hadden. Hen werd alsnog een mogelijk geboden om deel te nemen. Deze vrouwen waren woonachtig in de regio Noord-Holland of Flevoland. De vrouwen hadden niet gereageerd op hun oproep voor een uitstrijk alsmede niet op een herhaaloproep. Deze niet-deelneemsters kregen tussen eind 2006 en medio 2008 een thuistest opgestuurd om zodoende zelf, thuis slijmvlies van de baarmoedermond te kunnen afnemen voor nader onderzoek in het laboratorium. Na zenden van het zelf-afgenomen materiaal naar het laboratorium (VU medisch centrum Amsterdam) werden de zelf-afgenomen materialen onderzocht met behulp van de HPV test. Indien hrHPV aangetoond werd in het zelf-afgenomen materiaal, dan kregen de vrouwen het advies om naar de huisarts te gaan voor het laten maken van een uitstrijkje. Wanneer vervolgens abnormale cellen werden gevonden in het uitstrijkje, werden de vrouwen naar de gynaecoloog doorverwezen.

Het doel van het onderzoek was om na te gaan of niet-deelneemsters wel op de thuistest zouden reageren en bereid zouden zijn om, indien zij hrHPV positief getest zouden zijn, zich verder te laten onderzoeken door een huisarts/gynaecoloog. Tevens wilden we analyseren of de thuistest met HPV test geschikt was om CIN laesies te kunnen detecteren.

Het onderzoek wees uit dat het aanbieden van de thuistest aan niet-deelneemsters een goede manier is om niet-deelneemsters alsnog te laten deelnemen aan het BVO BMHK, want 30% van deze vrouwen deed mee aan het thuistestonderzoek. Dit zou betekenen dat bij het landelijk invoeren van de thuistest aan niet-deelneemsters, de dekkingsgraad van het BVO BMHK met zo'n 7% (=30% van 23%) zou kunnen toenemen. Wij kunnen hieruit concluderen dat de thuistestmethode voor een deel van de vrouwen de factor tot niet-deelname aan het BVO BMHK wegneemt.

Van de 30% van de vrouwen die deelnam aan de thuisteststudie, bleek 9,3% geïnfecteerd te zijn met hrHPV. Ongeveer 90% van deze vrouwen zijn naar de huisarts geweest voor het laten maken van een uitstrijkje. Iets meer dan een kwart van deze vrouwen hadden een afwijkend uitstrijkje en werden geadviseerd om naar de gynaecoloog te gaan voor verder onderzoek. Ongeveer 94% van deze vrouwen gaf hieraan gehoor. In totaal hadden 61 vrouwen CIN2 (matige dysplasie), 144 vrouwen CIN3 (ernstige dysplasie), en 13 vrouwen baarmoederhalskanker. Als we deze getallen

afzetten tegen de getallen onder vrouwen die regulier deelnemen aan het BVO BMHK, dan zien we dat niet-deelneemsters significant meer afwijkingen hebben dan vrouwen die recent een uitstrijkje hebben laten maken. M.a.w. de groep niet-deelneemsters kent inderdaad een verhoogd risico voor het ontwikkelen van baarmoederhalskanker.

De thuistest is in twee fasen uitgevoerd, met als belangrijkste verschil tussen de twee onderzoeken het type thuistest en de geboortejaren. Het eerste onderzoek betrof een thuistest welke een spoeling van de baarmoedermond uitvoerde om cellen van de baarmoedermond en vagina te verzamelen (***hoofdstuk 3***). Het tweede onderzoek maakte gebruik van een borsteltje om cellen van de baarmoedermond/vagina te verzamelen (***hoofdstuk 4***). Het tweede onderzoek is uitgevoerd onder vrouwen welke 1 jaar later waren uitgenodigd voor het laten maken van een uitstrijkje dan de vrouwen uit het eerste onderzoek. Het betrof derhalve een opschuiving van 1 jaar in geboortejaren. Vergelijking van de gegevens van beide fasen van het onderzoek toonden dat het type thuistest geen verschil maakt voor het uiteindelijke resultaat van het aanbieden van een thuistest; de vrouwen in beide onderzoeken waren even bereid (zo'n 30%) om deel te nemen aan het thuistest onderzoek. Tevens werd een gelijke hoeveelheid CIN laesies gevonden in beide fasen van het onderzoek.

Door het samenvoegen van de onderzoeksgegevens van de twee fasen van het onderzoek werden aanvullende analysen mogelijk, namelijk of etniciteit, uitstrijk-voorgeschiedenis en leeftijd van de vrouw invloed hebben op wel/niet deelname aan een thuistestonderzoek en de kans op het hebben van CIN laesies (***hoofdstuk 5***). Het analyseren van de gecombineerde onderzoeksgegevens per etniciteit toonde dat in Nederland wonende "Westerse" vrouwen (Europa, Australië, Nieuw-Zeeland, Canada en VS) significant meer meedoen aan het thuistestonderzoek dan in Nederland wonende "niet-Westerse" vrouwen (Azië, Afrika, Midden-Oosten, Midden- en Zuid-Amerika). Verder hadden ook de leeftijd en hun uitstrijk-voorgeschiedenis invloed op de deelnamebeslissing; jongere vrouwen deden meer mee dan oudere vrouwen en het deelnamepercentage onder vrouwen die nog nooit hadden deelgenomen aan het BVO BMHK was hoger dan onder vrouwen die weleens eerder een uitstrijkje hadden laten maken binnen het BVO BMHK. M.a.w. een belangrijke groep vrouwen die nooit een uitstrijkje hebben laten maken, wordt bereikt met de thuistest methode. Deze bevindingen waren gelijk voor beide fasen van de studie. De kans op laesies werd alleen beïnvloed door hun beslissing om wel/niet deel te nemen aan het BVO en niet door etniciteit of leeftijd.

In ***hoofdstuk 6*** analyseerden we de vrouwen die behandeld waren voor hun hooggradig CIN laesie. We keken of er een relatie was tussen de verschillende hrHPV typen en de kans op het herkrijgen van de laesie. Hieruit bleek dat vrouwen die geïnfecteerd waren met hrHPV type 16, het meest voorkomende HPV type, de grootste kans hadden op wederom een CIN laesie binnen een paar jaar. Hieruit kunnen we concluderen dat de gynaecologen extra aandacht dienen te geven aan deze groep vrouwen.

8.6 Conclusie

Wij hebben laten zien dat de thuistest methode geschikt is om niet-deelneemsters van het BVO BMHK te kunnen bereiken. PROHTECT thuistestonderzoek laat zien dat het aanbieden van een thuistest met een hrHPV test in het laboratorium, een uitvoerbare en efficiënte benadering is die de effectiviteit van het bevolkingsonderzoek naar baarmoederhalskanker kan verbeteren. Ons onderzoek heeft dit met name getest onder de groep vrouwen die niet hadden gereageerd op een uitnodiging voor het laten maken van een uitstrijkje binnen het BVO BMHK. Ruim 30% van de niet-deelneemsters bleek wel deel te willen nemen aan het BVO BMHK wanneer hen de mogelijkheid van een thuistest werd geboden. In deze groep niet-deelneemsters vonden we uiteindelijk een hoger dan normaal percentage voorloperstadia alsmede 13 gevallen van baarmoederhalskanker. We concluderen dat de thuistestmethode en het daarbij behorend protocol geschikt is om hooggradige voorloperstadia te detecteren en daarmee baarmoederhalskanker te voorkomen, juist in de groep vrouwen die onvoldoende deelneemt aan het BVO BMHK.

In de toekomst zou deze thuistestmethode uitermate geschikt zijn om breed te implementeren in het BVO BMHK. Niet alleen niet-deelneemsters, maar alle vrouwen die dit wensen zouden dan in aanmerking moeten kunnen komen voor de thuistest. Hiermee kan de dekkingsgraad toenemen, het aantal gevallen van CIN laesies afnemen en de kwaliteit van het BVO BMHK verbeteren. Verder kan dit de werkdruk van de huisarts doen afnemen, omdat alleen de HPV-positieve vrouwen zullen worden geadviseerd om naar de huisarts te gaan. Recente onderzoeken hebben laten zien dat de HPV test in combinatie met een thuistest een even betrouwbaarder resultaat (dwz detectie van baarmoederhalskanker en de ernstige voorloperstadia daarvan) geeft als het klassieke uitstrijkje of het uitstrijkje met een HPV test. Deze resultaten rechtvaardigen om in de toekomst ook de HPV thuistest als onderdeel van het BVO BMHK in Nederland te implementeren.

List of Publications

1. Stooker W, **Gök M**, Sipkema P, Niessen HW, Baidoshvili A, Westerhof N, Jansen EK, Wildevuur CR, Eijsman L. “Pressure-diameter relationship in the human greater saphenous vein”. *Ann Thorac Surg. 2003 Nov;76(5):1533-1538*.

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Arash en ***Maikel***, what can I say? Volgens mij, hebben we iets te veel lol gehad, soms (letterlijk) met de tranen rollend over onze wangen. Het was niet goed dat we met z'n drieën op een kamer zaten. Maar buiten werktijden zijn er geen grenzen, natuurlijk. Vanzelfsprekend, gaan we gewoon verder met contact onderhouden. *Oke, that's it!.....* (vul de rest maar zelf in, Maikel).

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Curriculum Vitae

Murat Gök werd op 10 juli 1974 geboren te Zaandam (tegenwoordig Zaanstad). Na de basisschool begon hij aan het middelbaar algemeen voortgezet onderwijs (MAVO) om vervolgens na 4 jaar te beginnen aan het hoger algemeen voortgezet onderwijs (HAVO) te beginnen. Nog niet genoeg gekregen van leren, is hij na 2 jaar begonnen aan het voorbereidend wetenschappelijk onderwijs (VWO). Na 2 jaar het diploma gehaald te hebben, is hij in 1995 geneeskunde gaan studeren aan de Vrije Universiteit te Amsterdam. Dit betekende voor hem het einde van hardrocktijdperk en begin van het dragen van iets nettere overhemden en schoenen.

Tijdens zijn studie heeft hij meerdere stages in het buitenland gelopen en bijna 2 jaar onderzoek gedaan op de afdeling cardiochirurgie, VU medisch centrum. Hieruit vloeide voor hem een artikel voort (tweede auteur). Hij kreeg hierdoor de smaak te pakken voor wetenschappelijke onderzoeken.

In 2004 behaalde hij zijn artsenexamen. Hierna heeft hij achtereenvolgens negen maanden als AGNIO op de afdeling cardiochirurgie en negen maanden op de afdeling longziekten gewerkt, om vervolgens eind 2005 op de afdeling pathologie, VU medisch centrum, te belanden als arts-onderzoeker o.l.v. prof. dr. C.J.L.M. Meijer. De resultaten zijn beschreven in dit proefschrift.

Hij woont nog steeds in Zaandam, samen met zijn vrouw Esengül Boz en hun dochter Lara (2006).

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