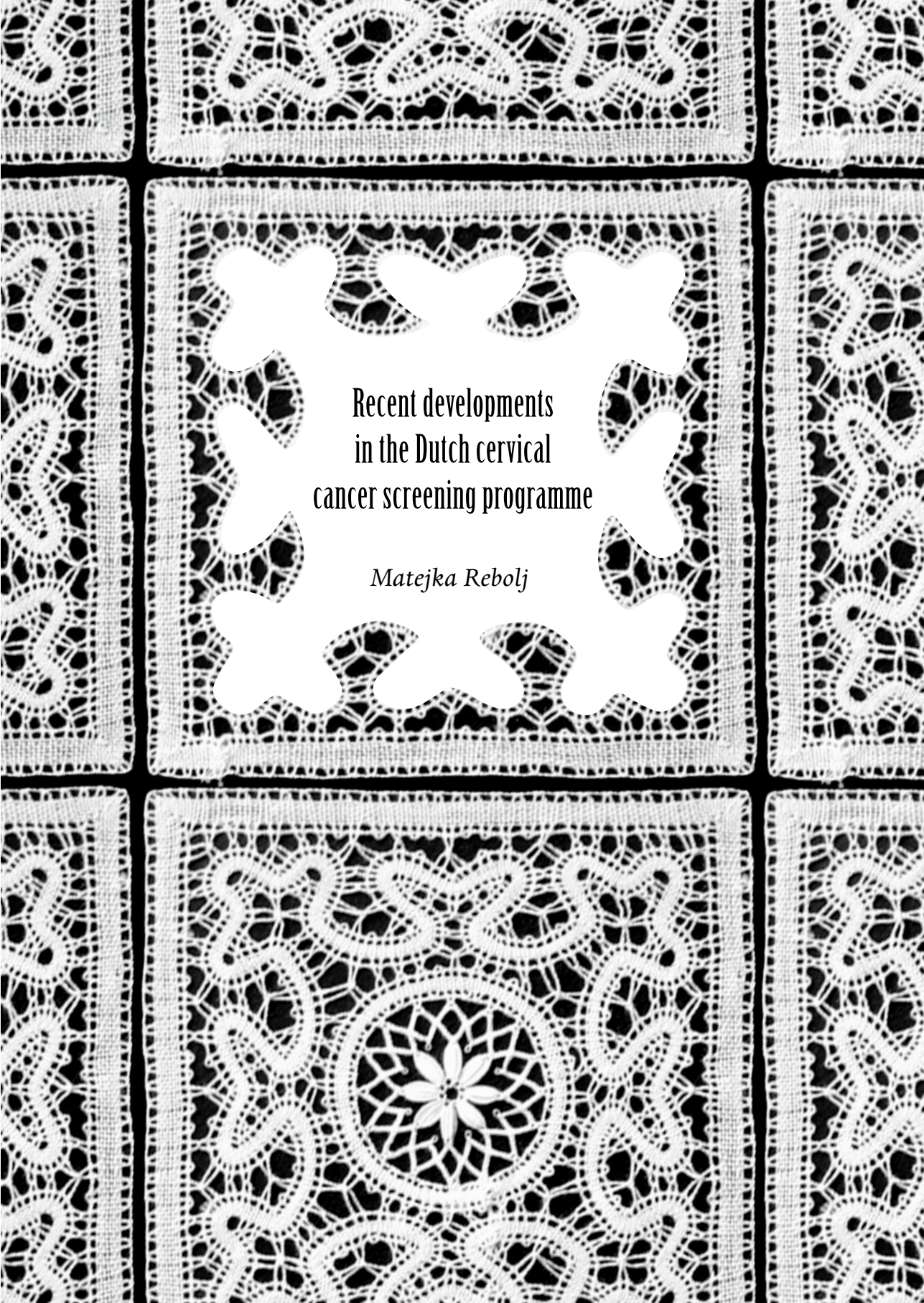

Recent developments in the Dutch cervical cancer screening programme

Matejka Rebolj

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Recent Developments in the Dutch Cervical Cancer Screening Programme

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For those who matter

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CHAPTER 1. General Introduction

1.1. Cervical cancer incidence and mortality

Worldwide, cervical cancer is the second most common female malignancy, diagnosed in 500,000 women each year, while 275,000 die from it.[1] Without prevention, the peak incidence occurs at a relatively young age, between 40-55 years,[1, 2] when women are still active on the labour market and have young children.

While cervical cancer is the leading cancer-related cause of death[1] and the second most common cancer in women in developing countries (incidence rates ≥30 per 100,000),[3] it became much less common in developed countries in the recent decades. In the Netherlands, the incidence and mortality have been decreasing for decades (Figure 1-1). In 2003, cervical cancer was newly diagnosed in 584 women (World standardized incidence rate (WSR): 4.9 per 100,000 women) and 214 women died from it (WSR: 1.4 per 100,000 women).[4]

Inter-country differences in cervical cancer incidence are caused by differences in determinants and in access to preventive measures. The prevalence of the Human Papillomavirus (HPV) infection, the necessary factor in the development of cervical cancer,[5] is generally higher in developing countries.[6] Differences in the host factor, e.g. more common malnourishment and the prevalence of other infections in the developing countries,[7] may also play a role. These countries typically do not offer population-wide cervical cancer screening facilities, which require a high level of organization and adequate health care resources.

1.2. The epidemiology of Human Papillomavirus infection and its role in the aetiology of cervical cancer

The association between HPV and cervical cancer is among the strongest ever observed for human cancer, and is considered causal but not sufficient for developing cervical cancer.[3, 5, 8] The usual mode of transmission of anogenital types of HPV is through sexual contact.[3] HPV infection is most common when women are most sexually active. For example, HPV can be detected in 15-25% of women below age 30.[9-13] After age 30, the prevalence decreases to 3%-7%.[9, 12, 14-17] In some countries, a second peak around age 50 has been described, though it is much lower than among women below age 30.[16, 18-21] It is estimated that 50-80% of women will be infected with HPV at least once in their lifetime.[22]

As the immune system is usually capable of clearing the HPV without any medical intervention,[25-28] most HPV infections are transient. Eventually only up to 20% of infections cause preinvasive cervical neoplasia.[29] While the lesions are still preinvasive, HPV clearance leads to spontaneous regression of the majority of these lesions. Invasive cancer, on the other hand, is a rare consequence of an infection with one or more of the high-risk, or oncogenic, types of HPV.[5, 8, 30] Oncogenic HPV types are defined as those that could be identified in cervical cancer: types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82, and probably also 26, 53, and 66.[31]

1.3. Secondary cervical cancer prevention

Cervical cancer is preventable. It is preceded by a long asymptomatic but screen-detectable preinvasive stage lasting more than 10 years on average[32-34] which is identifiable as cervical intraepithelial neoplasia grades 1, 2 or 3 (CIN 1-3), ranked by severity of the abnormality.[35, 36] The majority of these lesions, about 75%, would never progress to cancer in absence of treatment.[32, 37] Because it is at present not possible to distinguish between the progressive and non-progressive lesions, women with preinvasive lesions, especially the high-grade ones (usually defined as CIN 2+), are usually treated. Outpatient CIN treatment has a very high success rate. It reduces the risk of progression to cancer by 95%.[38]

In the past decades, the predominant mode of cervical cancer prevention has been secondary prevention – screening for preinvasive lesions. Historically, the most important screening tool has been cytology using the Pap smear, whereas recently also HPV testing is gaining in importance.

The Pap smear

The presence of cervical abnormalities is primarily screened for with a Pap smear. The Pap smear was developed in the 1940's[39] and was adopted in developed

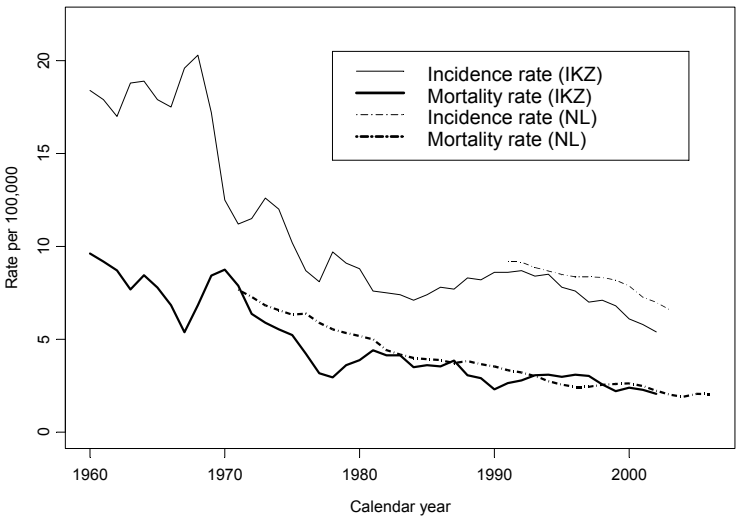

Figure 1-1. Incidence and mortality rates in the Netherlands per 100,000 women (European standardized rate).[4, 23, 24]
IKZ = Cancer Registry South; NL = The Netherlands.

Table 1-1. Cytological (Pap smear) and histological classification applying to cervix uteri.

HISTOLOGY									
Description (CIN classification)[35, 36]	Inadequate	Normal		Borderline dysplasia/ Atypia (CIN 1)	Mild dysplasia (CIN 1)	Moderate dysplasia (CIN 2)	Severe dysplasia (CIN 3)	Carcinoma in situ (CIN 3)	Carcinoma
CYTOLOGY									
Pap classification[49]	Pap 0	Pap 1		Pap 2	Pap 3a1	Pap 3a2	Pap 3b	Pap 4	Pap 5
CISOE-A†[49]	S0 00 E0	S1 01 E1-2	S1 02 E1-2	S2-3 03 E3	S4 04 E4	S5 05 E5	S6 06 E6	S7 E7	S8-9 07-8 E9
Bethesda 2001‡[50]	Unsatisfactory for evaluation	Negative	Atrophy	ASC-H		HSIL			Squamous cell carcinoma
				ASC-US	LSIL				
				AGC	AGC favor neoplastic			AIS	Adenocarcinoma

†KOPAC-B, i.e. the classification in use in the Netherlands since 1996: S=squamous epithelium (1=normal; 2=abnormal squamous epithelial cells; 3=atypical squamous metaplasia; 4=mild dysplasia; 5=moderate dysplasia; 6=severe dysplasia; 7=carcinoma in situ; 8=microinvasive carcinoma; 9=invasive squamous carcinoma); 0=other abnormalities / endometrium (1=no other abnormalities; 2=epithelial atrophy; 3=atypical repair reaction; 4=mildly atypical endometrium; 5=moderately atypical endometrium; 6=severely atypical endometrium; 7=adenocarcinoma endometrium; 8=metastasis malignant tumor); E=endocervical columnar epithelium (1=normal; 2=no endocervical cells present; 3=some atypical endocervical cells; 4=mildly atypical endocervical epithelium; 5=moderately atypical endocervical epithelium; 6=severely atypical endocervical epithelium; 7=adenocarcinoma in situ endocervical epithelium; 9=adenocarcinoma endocervix). ‡ASC-US atypical squamous cells of undetermined significance; ASC-H atypical squamous cells cannot exclude HSIL; AGC atypical glandular cells; LSIL low-grade squamous intraepithelial lesion; HSIL high-grade squamous intraepithelial lesion; AIS adenocarcinoma in situ.

countries in the subsequent decades as a mass screening tool. The Pap smear is a cytological sample of exfoliated cells collected directly from the cervix uteri. Primarily taken by a nurse, a general practitioner or a gynaecologist, it is thereafter evaluated under a microscope by a cytotechnician or a pathologist. Subsequently, cytologycally identified morphological changes are diagnostically confirmed by a gynaecologist applying colposcopy-guided histological sampling, which is evaluated in a laboratory by a pathologist. Table 1-1 relates several commonly used cytological classifications[40] with those for the histological outcomes.

The Pap smear induces a certain degree of over-treatment, as a proportion of lesions that are detected and treated would spontaneously regress.[41] There has been no randomized controlled trial to assess the decrease in incidence and mortality from cervical cancer due to screening. Several important epidemiologic studies nevertheless observed that the widespread introduction of Pap smear screening accelerated the already existing downward trends in the incidence and the mortality from cervical cancer.[42, 43] These shifts in trends were strongest in countries with more intensive screening schedules, and in the age groups that were targeted by the programmes (as opposed to younger and older women in whom very little screening was done).

New technologies: the HPV test

The identification of the causal role of the HPV in the development of cervical abnormalities allows secondary prevention through HPV detection, as well primary prevention through HPV vaccination. Much effort has been invested into improving the tools for HPV detection.[44] At present, the most widely used types of HPV tests are the Hybrid Capture II (HC II) and the Polymerase Chain Reaction (PCR) tests. Compared to the Pap smear, the HPV test detects an (active) HPV infection. The currently available HPV tests offer better test reproducibility and sensitivity, but are less specific for progressive conditions, and at present more expensive.[45] In recent years, the accumulated epidemiological evidence and the technological advancements have fuelled a discussion on whether and in what way the HPV test could or should complement or substitute the Pap smear.[45-47] To date, three potential applications of HPV testing to enhance cervical cancer screening and diagnosis have been proposed: in primary screening, in triage of low-grade cytological abnormalities, and in follow-up after treatment of preinvasive lesions.[45, 48] However, because HPV testing has long been an emerging technology, high-quality data with long-term follow-up is just beginning to become available.

1.4. Cervical cancer screening in the Netherlands

In the Netherlands, Pap-smear screening became widespread in the 1970's when 3 pilot screening programmes were started in Nijmegen, Rotterdam and Utrecht. Similar programmes were soon adopted in other regions but were stopped by mid-1980's when decentralized programmes were introduced instead. It was soon established that these were not performing well: the coverage was unsatisfactory, and the burden of false-positive screening results was high.[51-53] In 1991, the Ministry of Health called for the programme to undergo substantial changes as a condition for centralized financing.[54] Several professional groups

involved in screening (pathologists, general practitioners, gynaecologists, cancer registries, municipal health authorities, epidemiologists) debated the possible alternatives. As part of this process, a comprehensive ex-ante cost-effectiveness analysis performed by the Department of Public Health of the Erasmus MC (before 2003 Erasmus University)[55] played an important role in the reconsideration of the age range and the screening interval. In 1993, the Dutch Health Insurance Council issued the main programme guidelines[56] that were followed in 1996 by the national guidelines for general practitioners,[57] in 1997 for pathologists[49] (revised in 2006)[58] and in 1998 for gynaecologists.[59] Screening according to the new guidelines, described in detail together with the outcomes in Chapter 2 of this thesis, was implemented nationally in 1996 at an estimated yearly cost of approximately €30 million.[60]

1.5. The role of programme evaluation in the Netherlands

In contrast with the situation before 1996, regional and national monitoring and evaluation became a component of the programme. Regular monitoring is required both to identify and solve specific bottlenecks that are impeding an efficient and high-quality service delivery, and more generally to ensure that the public finances continually invested in the programme are spent in a responsible way.

Throughout this period, national monitoring and evaluation has been performed by the Department of Public Health of the Erasmus MC in Rotterdam. Several periodic national reports have been published: regarding the years 1994[61] (the last year before the stepwise implementation of the new guidelines, serving as the base to measure subsequent changes), 1996 and 1997,[62] 1998-2001,[60] 2002,[63, 64] 2003,[63, 65] 2004,[66] 2005[67] and 2006,[68] as well as an ex-post cost-effectiveness analysis,[69] alongside several international publications on specific topics.[41, 70-80]

The data source for the national evaluation

The data used for the evaluation was periodically retrieved from the Dutch nation-wide network and registry of histo- and cytopathology (PALGA). From 1990 onwards PALGA in principle achieved national coverage of pathology laboratories.[81] Rather than the evaluation of screening activities, its primary purpose was to support physicians in making everyday treatment decisions for their patients. As such, especially for the earlier registration years, it does not allow the evaluation of all aspects of the screening programme. Nevertheless, it offers information on all cytology and histology testing performed, regardless of the technique, setting and reason. Because for every test registered in PALGA the woman's (coded) identification, the date, and the topographic and morphologic codes (Thesaurus-compatible codes for histologic tests, and CISOE-A codes for smears[49]) are known, screening and diagnostic histories can be observed for all women with at least one test performed on their cervix uteri. Moreover, the use of PALGA for cervical cancer screening evaluation has increased and eventually led to improvements of the registry for this purpose. Only few other countries have established a similarly comprehensive data source that enables a comprehensive evaluation of the screening activity.[82]

In a collaboration between Prismant (the PALGA data manager) and the Department of Public Health of the Erasmus MC, all PALGA records with cervix uteri as topography are retrieved and interpreted within the so-called PALEBA project.[83] At present, the PALEBA/PALGA file contains the data retrieved until 31st March 2007, with almost 20 million records for at least 5 million screened women, and offers 17 years of follow-up with complete coverage.

1.6. Research questions in the thesis

The goal of this thesis is to give a comprehensive overview of the – short-term as well as long-term – outcomes of the changes in cervical cancer screening in the Netherlands since 1996. Other goals were to evaluate the need for continued cervical cancer screening in women with several consecutive negative smears by age 50, and to explore the possible role of HPV testing as a future technique for cervical cancer prevention. The research can be summarized as addressing the following 5 research questions.

1. What were the short-term effects of changes in screening programme protocols and guidelines since 1996?

The changes concerned both the medical and the organisational aspects. Their goal was to streamline the screening organisation and process, and to improve the balance between screening effectiveness, negative side effects, and costs. These goals are reflected in several short-term screening process indicators: the screening coverage, the proportion of women screened with positive results, the completeness of follow-up, and the yearly number of smears made. In Chapter 2, the changes in these indicators were evaluated when the first 5-year screening round under the new protocols and guidelines was completed.

2. Did the sensitivity of the Pap smear decrease after a broader definition of a negative smear?

In population-based screening of apparently healthy individuals, test specificity, i.e. the proportion of truly non-diseased persons who are so identified by the screening test,[84] should be high.[85] This was not the case in the Netherlands before 1996, when in each screening round more than 10% of all smears were classified as abnormal.[51] In response, the threshold for minimum relevant smear abnormality (requiring short-term follow-up) was elevated so that inflammation without concurrent dysplasia was no longer classified as abnormal.[49] This caused a drop in the frequency of smears classified as abnormal by about 80%.[86] A higher cut-off point for test positivity will decrease the sensitivity of the screening test, i.e. the proportion of diseased persons in the population who have a positive screening test.[84] However, the sensitivity is very complex to estimate empirically[87] because the majority of the screened population is screened with negative results and is not subjected to subsequent diagnostic testing – their true disease status is therefore unknown. For this reason, test sensitivity is in practice judged through the interval cancer incidence rate, i.e. the incidence of cervical cancer after a negative smear. The observed change in this rate for the Dutch cervical cancer screening programme since 1996 is presented in Chapter 3.

3. Can screening be ceased for women with several negative smears by age 50?

Cervical cancer screening is an effective, yet compared to e.g. breast cancer screening a relatively costly preventive health

intervention; it also induces anxiety in women.[43, 88, 89] Identification and exclusion of subgroups at negligible risk create an opportunity to decrease this burden without measurably decreasing the overall effectiveness of the screening programme. Several authors argued that this is the case with well-screened women who at the age of around 50 had several consecutive negative smears.[90-92] For example in the Netherlands, this would apply to about 50% of all women screened around age 50,[93] and would translate into a yearly reduction of at least 65,000 smears, or close to 15% of all programme smears.[68, 69] The age limit for screening of women with several consecutive negative smears continues to spawn interest until today,[94] as the discussion could not be closed because of lack of suitable data. Studies so far focused on the detection of CIN.[18, 90-92, 95-97] Because there is strong evidence that CIN has a higher progressive potential at older compared to younger ages,[34] the comparison of the frequency of detection of these lesions as a proxy for comparing the risk for cancer in younger and older women is questionable. In Chapter 4, we reassessed the need for continued screening by comparing the incidence of cervical cancer.

4. What is the negative predictive value of the HPV test compared to the Pap smear in primary screening?

One of the possible applications of an HPV test is to use it as a replacement of the Pap smear in primary screening.[45] Whether this is worthwhile cannot be simply calculated from cross-sectional relative sensitivity and specificity data. Rather, screening programmes would need to be adjusted to achieve the optimal gain from the different detection profile of an HPV test. Depending on how much more sensitive HPV testing is for the underlying cancer precursors compared to the Pap smear,[45] and how much earlier it detects them,[98-100] one of the adjustments would be lengthening the screening interval. To investigate this issue, women were screened within a multi-centric European project with the combination of the Pap smear and the HPV test, and thereafter followed for several years. We evaluated the risk for CIN 3+ associated with three different combinations of these two tests (cytology alone, HPV alone, and a combination of both). Our findings on the most optimal procedure, with their implications for the future of cervical cancer screening are discussed in Chapter 5.

5. What is the optimal use of HPV testing in triage of women with a persistent low-grade abnormal smear?

Another possible application of HPV testing is in triage of low-grade cytological abnormalities.[101] Women with low-grade cytological abnormalities in screening smears are in general referred to a gynaecologist after one (e.g. in the Netherlands) or more abnormal follow-up smears.[49, 102, 103] Ten to 40% of these women have a CIN 2+ lesion,[104-109] and 60-90% do not. About one-half have a detectable HPV infection.[104-109] In a trial described in Chapter 6, we explored the safety of selecting only HPV-positive women for a referral to a gynaecologist. In Chapter 7, we additionally explored the optimal timing of HPV testing in the group of women with a persistent low-grade abnormal smear.

CHAPTER 2. Monitoring a National Cancer Prevention Programme: Successful Changes in Cervical Cancer Screening in the Netherlands

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2.1. Abstract

The success of screening, an important cancer prevention tool, depends on the quality and efficiency of protocols and guidelines for screening and follow-up. However, even centrally organised screening programmes such as the Dutch cervical screening programme occasionally show problems in performance. To improve this programme, the screening scheme, follow-up, administration and financing protocols and guidelines were thoroughly changed in 1996. This study evaluates the consequences for the performance of the national programme. The five-year coverage rate, the proportion of screened women sent to follow-up, follow-up compliance and duration, and the yearly number of Pap smears before and after the changes in 1996 were compared. Five-year coverage increased substantially in the added target age groups (30-34, and 54-60 years); in the old target age group (35-53 years) it remained around 80%. The percentage of screened women sent to follow-up decreased from almost 19% to 3% per screening round, due to a more restrictive use of the Pap 2 classification, and an evidence-based cessation of follow-up of negative smears without endocervical cells. Follow-up compliance has improved, and the average time until a woman is either referred or rejoins the regular screening schedule, has become shorter. The total number of smears, a strong determinant of screening costs, has decreased by 20% primarily due to the changed follow-up recommendations. In conclusion, the 1996 changes in protocols and guidelines, and their implementation have increased the coverage and efficiency, and decreased the screening-induced negative side effects.

2.2. Introduction

Screening is an important cancer prevention tool. In case of cervical, colorectal and breast cancer it is recommended[110] and adopted as national programmes in various countries.[111-113] Depending on the outcomes of ongoing trials,[114-116] more screening programmes (for prostate, lung and ovary cancer) may in the future be offered to the general population. The success of screening depends on the quality and implementation of protocols and guidelines for primary screening and follow-up. Often, the balance between prevented mortality from cancer, and the screening-induced costs and negative side effects (e.g., higher morbidity, unnecessary treatments and raised anxiety due to false-positive results) is fragile.[89, 117] Also, people may be screened too frequently, while others are not screened at all.[118, 119] Further, new screening tests become available occasionally,[120] and these require thorough evaluation before they can be incorporated into a screening programme. Screening therefore requires regular monitoring and adaptation in order to keep the programme effective and efficient.

Since the 1980's, cervical cancer screening with the Pap smear has been offered to the population in the Netherlands through an organised programme. Pap smear is a non-invasive test and aims to prevent cancer deaths by treating pre-invasive and early invasive disease. Like in the UK in the 1980's,[121-123] the evidence gathered in the Netherlands in the early 1990's pointed towards a suboptimally performing programme, in terms of both the organisation and the efficiency of screening of the target population.[51, 53, 55, 78] In 1993, the Ministry of Health called for an immediate inquiry into possible solutions.[54] Subsequently, new protocols and guidelines regarding the screening and follow-up schemes, administration and financing were implemented nationally in 1996 (Appendix, Chapter 2.6.).[56] The new screening and follow-up schemes were agreed upon in a consensus meeting of the five professional groups implementing cervical cancer screening (pathologists, gynaecologists, general practitioners (GP), regional cancer registries, and local health authorities) together with epidemiologists and Ministry of Health representatives. Much emphasis was devoted to assuring that the guidelines would be adhered to.[124]

Seven main implemented solutions can be distinguished. First, since 1996 the programme covers the whole country. Financing and coordination are managed centrally. The programme is implemented locally through special regional centres operating in a covenant, i.e. as financially binding associations of the five professional groups listed earlier. Second, all non-attendees to the first invitation are systematically sent reminders. Third, reimbursement of preventive smears taken outside of the regular screening schedule, which are considered ineffective and may result in unnecessary diagnostic and treatment procedures,[78, 125] has been abolished. Fourth, the screening interval was increased from 3 to 5 years in a broader target age group (30-60 instead of 35-53 years).[55] Fifth, new pathology guidelines[49] aimed to improve the specificity of the Pap smear by downgrading the considerable number of smears with sole morphocytological signs of inflammation and/or presence

of specific microorganisms from a borderline (Pap 2) to a negative (Pap 1) smear. Sixth, the new pathology guidelines aimed to limit the maximum duration of follow-up of borderline (Pap 2) and mildly (Pap 3a1) dyskaryotic smears (BMD) before the final referral or normal screening recommendation. Seventh, an amendment from 2002 ceased to advise a repeat Pap smear to negative smears lacking endocervical cells (Ecc-).[73, 126]

To assess improvement in the Dutch cervical cancer screening programme after the completion of the first 5-yearly screening round under the new protocols and guidelines, we will evaluate the impact of these changes on the five-year coverage rate, the proportion of screened women sent to follow-up, follow-up compliance and duration, and the yearly number of smears.

2.3. Material and Methods

Performance of the new protocols and guidelines is based on programme smears from 2003. The exception is the follow-up compliance, which is instead based on programme smears from 1999 in order to have available data for a 4-year follow-up period. This performance of the current programme is compared with the situation before 1996, which is based on programme smears from 1994.

Information on all cervix uteri cytological and histological tests in the Netherlands registered until 31st March 2004 was retrieved from the nation-wide network and registry of histo- and cytopathology ("PALGA"). From 1990 onwards all pathology laboratories were linked to this registry.[81]

PALGA identifies a woman through her birth date and the first four letters of the (maiden) name. In order to correct for occasional false matches in this identification,[127] we excluded the 0.5% most common surnames.[76] This applies to the results in Tables 1, 3, 4 and consequently 6, and excludes about 30% of women from the PALGA. Age is defined on 1st December of the analysed year, in agreement with the definition of age at invitation as used in the screening programme.

For all registered cervical smears, PALGA enables recoding of the reason for smear-taking into programme screening, spontaneous screening, medical complaints, or follow-up. This information is missing for 52% and 10% of the primary smears in 1994 and 2003, respectively. A programme smear is defined as any smear in PALGA that was primary and taken in the calendar year (or the first three months thereafter) in which the woman was eligible for the programme given her birth year.

Five-year coverage rates are calculated by comparing the number of women who had at least one smear taken for any reason (as counted from PALGA), with the estimated number of women at risk (i.e., with a cervix) alive on 1st January of the analysed year. The number of women at risk were estimated with the data obtained from the Dutch Central Bureau of Statistics,[23] decreased by the estimated number of women with their cervix removed by a hysterectomy.[128] Further, we excluded the 0.5% common surnames both in the numerator and the denominator. Because the latter cannot be obtained from any official statistics, we approximated the fraction of women remaining in the population at risk after the exclusion of 0.5% most common surnames by observing the fraction of tests remaining in PALGA after the same exclusion. The implicit assumption here is that the screening behaviour and the commonness of surnames are not associated.

A screening episode is defined as starting with a primary test and eventually followed by secondary tests (smears or biopsies). Follow-up or secondary tests are tests made within 4 years of an abnormal smear, an inadequate quality smear, or a non-negative biopsy after which follow-up has not yet been completed according to the guidelines. All other tests are seen as primary tests starting the episode. An episode is finished either when follow-up is complete according to the current guidelines (e.g., two consecutive negative smears after a BMD smear, or three consecutive negative smears after treatment of histologically confirmed cervical intraepithelial neoplasia), or when there are no more tests registered in 4 years. The categorisation of smear results and the corresponding follow-up recommendations are presented in the Appendix (Chapter 2.6.).

Excess smears are defined as all smears that are taken in a certain period that do not contribute to the observed coverage in the target population. These may be due to e.g. more frequent screening than it is recommended, but also due to secondary (diagnostic) testing. The number of excess smears is presented for the relevant screening interval and 1,000 women per year; it is equivalent to (total yearly number of smears – number of smears needed yearly to reach the observed coverage) × 1,000 / number of women in the target group. The number of smears needed yearly to reach the observed coverage equals (the population at risk in the target age range × observed coverage) / recommended interval.

2.4. Results

Five-year coverage in the target population reached 77% in 2003 (Table 2-1), which is 12% points higher than the response to screening invitations, i.e. the attendance rate of 65% (data not shown). This difference is due to smear-taking outside the programme. The coverage in 1994 in the then targeted age group (35-53 years) was higher than in the newly targeted age group in 2003 (30-60 years). However, given that based on evidence it has been decided since 1996 that a larger age group is to be reached, the situation has improved. The coverage increased substantially in the added target age groups, while in the age groups targeted both before and after 1996 it remained at about the same level.

One percent of the programme smears was of inadequate quality for evaluation in 2003 (Table 2-2). Moderately dyskaryotic or worse abnormalities (>BMD) were found in 0.5% of programme participants, and further 1.8% of women had a BMD smear. The remaining women, 96.7%, had a negative smear.

Table 2-1. Five-year coverage rates in the Netherlands before (1994) and after (2003) the implementation of the new cervical cancer screening protocols and guidelines, for women aged 30-64, per age group.

Age group	2003	1994
30-34	68%	(53%)
35-39	77%	81%
40-44	81%	83%
45-49	81%	82%
50-54	81%	82%
55-59	79%	(59%)
60-64	74%	(23%)
Total	77%	82%†

()=Became part of the target age groups from 1996 onwards.
†Based on ages 35-54.

Table 2-2. Primary smear results in the organised cervical cancer screening programme before (1994) and after (2003) the implementation of the new protocols and guidelines, by type of follow-up recommendation.

Follow-up recommendation	2003	1994
No follow-up		
Due to a negative smear	84.8%	81.1%
Due to an Ecc- smear†	11.9%	7.3%
Follow-up		
Follow-up smear	1.8%	10.0%
Immediate referral	0.5%	0.3%
Repeat smear due to inadequate quality	1.0%	1.3%
Total	100%	100%

†Before 2002, the guidelines recommended Ecc- smears to be repeated after one year. Since January 2002, this recommendation has been withdrawn.

Compared with the situation in 1994, there are two interesting differences. First, there was a more than 80% decrease in the frequency of a follow-up smear advice. This was caused by the change in the definitions regarding the classification of sole morphological signs of inflammation. Second, immediate referral recommendation because of high-grade cytological abnormalities has almost doubled in frequency because since 1996 moderately dyskaryotic smears (Pap 3a2) also fall into this category (Appendix, Chapter 2.6.).

Among women with a 6-month follow-up smear advice (BMD smears), 74% received follow-up within 9 months, which we define as timely follow-up according to the guidelines (Table 2-3). Compared with 1994, the compliance with follow-up guidelines has increased substantially. There has also been an increase in longer-term follow-up (measured within four years).

Table 2-3. Compliance with follow-up after primary screening programme smears before (1994) and after (1999) the implementation of the new protocols and guidelines, by type of follow-up recommendation.

Follow-up recommendation	1999	1994
Follow-up smear		
Percentage of programme smears	2.1%	10.0%
– Of which with timely follow-up†	74%‡	44%‡
– Of which followed-up in 4 years*	90%	78%
– Of which with a biopsy (of *)	27%	14%
Immediate referral		
Percentage of programme smears	0.6%	0.3%
– Of which with timely follow-up†	85%	91%
– Of which followed-up in 4 years*	97%	97%
– Of which with a biopsy (of *)	94%	95%
Repeat smear due to inadequate quality		
Percentage of programme smears	0.8%	1.3%
– Of which with timely follow-up†	42%	45%
– Of which followed-up in 4 years*	85%	85%
– Of which with a biopsy (of *)	3%	18%

†Defined as 150% of the recommended follow-up interval, or 3 months for immediate referral. ‡First step of follow-up only.

The time in follow-up for these women until the final referral or a screening recommendation was shorter than in 1994 (Table 2-4). This difference is especially notable for women with a negative follow-up outcome: they were on average recommended to rejoin the normal screening schedule 12 months earlier than in 1994. Women with a positive follow-up outcome were on average referred to a gynaecologist 4 months earlier. Moreover, after on average 4.6 years, fewer women had no clear final follow-up recommendation in 1999 than in 1994. The average number of tests performed before the final either referral or a screening recommendation decreased by 10% (data not shown).

In the recent period, 85% of the women with a direct referral advice (>BMD smears) received follow-up in time (3 months), which increased to 97% within 4 years (Table 2-3). This compliance has not improved since 1994, but did also not worsen even though the new protocols on immediate referral include the moderately dyskaryotic smears (Pap 3a2). Among the 97% of followed-up women, 94% had at least one biopsy. For smears that need to be repeated due to inadequate quality the follow-up compliance has remained similar. However, contrary to recommendations these women were in 1994 more often referred and biopsied immediately instead of having a repeat smear taken.

In 2003, programme smears represented 67% of all smears, and those taken for medical complaints 13%. Spontaneous screening was small in volume at less than 2% of the total screening activity. Together with those smears for which no reason for smear-taking is given, primary smears add up to 91% of all smears (Table 2-5). Secondary smears account for 9% of all smears.

In total, 787,506 smears were taken in 2003, which represents an overall drop of 20% since 1994 (Table 2-5). Compared with 1994 and adjusted for population growth, the number of primary smears was about 2% lower in 2003. The decreased number of primary smears coupled with an increased coverage in a broad age range must have been associated with a longer average interval between consecutive primary smears. Indeed, both the frequency of very short screening intervals, as well as of the intervals of 2-4 years decreased (data not shown). The effect of the screening scheme protocol change is here intertwined with better adherence to these protocols. Moreover, there was less screening outside of the target ages, especially among women before their thirties (Table 2-6). Secondary smears dropped by 73% between 1994 and 2003 (Table 2-5). This drop was in approximately two-thirds due to how smears are evaluated with regard to inflammation (i.e., the Pap 2 definition change), and in the remaining third due to cessation of follow-up to Ecc- smears since January 2002.

2.5. Discussion

Since the 1996 changes the performance of the Dutch cervical cancer screening programme has improved considerably. With the same number of 7 smears per lifetime in a broader age range, the 5-year coverage in the added target age groups (30-34, and 54-60 years) rose substantially to 70% or above, with a loss of a few percent in the old target age group (35-53 years) where the coverage remained around 80%. This better coverage

Table 2-4. Final recommendation for women with a 6-month follow-up smear advice (to rejoin the normal screening schedule, or a referral to gynaecologist), and average duration until final recommendation, before (1994) and after (1999) the implementation of the new protocols and guidelines.

	Frequency		Average time (months)∫	
	1999	1994	1999	1994
Referred	31%	7%	10	14
Sent back to screening	32%	24%	25	37
Early histology†	4%	7%	5	12
Undetermined follow-up‡	24%	46%	n.a.	n.a.
Without any follow-up	9%	16%	n.a.	n.a.
Total	100%	100%		

Based on average follow-up time of 4.6 years per woman (range: 4.0 to 5.3 years). n.a.=not applicable. †Women with a histological result registered before it is expected according to the follow-up guidelines. ‡Women with follow-up, yet without test results suggesting a referral or a screening recommendation, and without early histology. ∫Time between the programme smear and the test that determined the final follow-up recommendation conforming to the guidelines.

was achieved with a 20% lower number of smears. The share of women sent to anxiety-causing follow-up decreased from 19% to 3% per screening round. Ninety percent of these women, an increase of 11% points, were followed-up after their non-negative screening smear. They spent a considerably shorter time in follow-up before they were either referred, or rejoined the regular screening schedule (Table 2-6). These results contribute towards gains in effectiveness and efficiency, and a reduction in screening-induced negative side effects and costs associated with cervical cancer screening in the Netherlands. Because before 1996 for slightly more than a half of the primary smears the reason for smear-taking was not known, in an additional analysis we included these smears. The pooled estimate of smear non-negativity rate in 1994 was 16% instead of 19% reported in Table 2-2, and the recent 3% still represents an 81% reduction since 1996 (instead of 84% reduction compared to 19%).

Coverage is a major effectiveness determinant of cervical cancer screening.[129, 130] In the age groups targeted both before and after 1996 (35-53 years) a small decrease in 5-year coverage rates was observed (Table 2-1). It appears that the lower frequency of invitations (one invitation every five instead of three years) has been counteracted by the beneficial effects of the 100% (i.e., nation-wide) invitation coverage and systematic reminders. Recent evidence[131] has shown that the uptake of screening is higher when the invitation is sent by the GP as opposed to the municipality. Further expansion of a GP invitational system is currently under review. The English experience with introducing graded financial incentives for the GPs to stimulate coverage has been positive,[103] though feasibility and acceptability of implementing such an instrument in the Dutch health care is not known.

Sending fewer women to follow-up, i.e. an increase in test specificity, has the potential of decreasing test sensitivity. Because of the low predictive value for (future) cancer of borderline dyskaryotic smears,[132, 133] and the long average screen-detectable preinvasive period for cervical cancer,[32-34] it is expected that when women are regularly screened, less intensive follow-up will only lead to a very small loss in program sensitivity. The CIN 3+ detection rates, i.e. the number of histologically confirmed cervical intraepithelial lesions grade 3 or higher per 1,000 women screened, increased from 4.4 to 5.4 (i.e., by 22%) between 1994 and 1999 (corrected for the extent of default to follow-up; data not shown). If anything, this does not point at lower sensitivity. However, even assuming no change in sensitivity, this increase was expected due to both an earlier start of programme screening (higher incidence and prevalence of CIN in young women), and a longer screening interval in 1999 than in 1994 (higher within-interval cumulative incidence of CIN 3). It is unknown to what extent changes in the detection rates are due to changes in the degree of over-diagnosis. A better indicator of the potential change in programme sensitivity, i.e. the interval cancer rate, is going to be examined in later analyses.

There is quite a diversity in how cervical cancer screening is organised in Europe,[111, 134] and its performance varies widely.[70] Compared to other countries with organized national programmes (Table 2-7), the Netherlands has been successful in limiting the number of excess smears while maintaining a high coverage rate. For example, even though the recommended screening interval is 5 years in Finland, the yearly number of smears is enough to screen each woman in the target age every 2.1 years, compared to every 4.7 years in the Netherlands. There are several reasons for this phenomenon. Contrary to Finland, procedures exist in the Netherlands (likewise in Sweden and England) that help sort out women with very recent smears. Next, smears taken outside of the regular screening schedule are in the Netherlands only reimbursed when the woman has medical complaints. Finally, Pap-smear screening in the Netherlands primarily takes place in GP practices, and much attention has been paid in the Dutch guidelines for GP practices to the frequency of smear-taking.[57]

The structure of follow-up advices given in the programme also differs among countries. While the Netherlands and Finland both have a low rate of inadequate smears, the sample inadequacy rate in England is close to 10%. This is one of the reasons why liquid-based cytology is considered a cost-effective option in England[141] and not in the Netherlands.[142] England and Finland have a high positivity rate of around 7%. The Netherlands and Sweden are both low with 2.3% and 1.5%, respectively. The fact that the bulk of the differences disappears if one compares the frequency of only highly positive programme smears is consistent with the view that the countries are using widely differing definitions of BMD abnormality. Therefore, the planned comparison of interval cancer rates before[76] and after the sharp decline in the proportion of BMD smears in the Netherlands will also be interesting from an international perspective.

The observed improvement in the Dutch cervical cancer screening programme was achieved in a short time. Implementation of protocols and guidelines is a complex process that depends on both the protocols and the guidelines themselves as well as on the context in which they are implemented.[143, 144] In the Netherlands, the new protocols and guidelines[49, 57, 59

Table 2-5. Numbers of primary and secondary smears (× 1,000), by screening age group, before (1994, adjusted for population growth between 1994 and 2003) and after (2003) the implementation of the new protocols and guidelines.

	Primary smears		Secondary smears		Total	
Age group	**2003**	**1994**	**2003**	**1994**	**2003**	**1994**
<30	28	106	4	26	32 (4%)	132 (13%)
30-60	666	598	61	214	727 (92%)	812 (83%)
>60	26	29	3	9	29 (4%)	37 (4%)
Total	720 (91%)	733 (75%)	68 (9%)	248 (25%)	788 (100%)	981 (100%)

Table 2-6. Summary: effectiveness, screening-induced side effects, and efficiency indicators before and after the implementation of the new protocols and guidelines in 1996.

Process indicator	**After 1996**	**Before 1996**
Effectiveness		
Coverage in the target age group[†]	77%	82%
Overall compliance to follow-up[‡]	90%	79%
Screening-induced negative side effects		
Proportion of women with a follow-up advice	3%	19%
Average time in follow-up before sent back to screening (women with a follow-up smear advice)	25 months	37 months
Efficiency		
Number of excess smears per year per 1,000 women in the target age group[∫]	76	261
Number of smears per 1,000 women aged 20-29, per year	33	111

[†]After 1996: 30-64 years, before 1996: 35-54 years. Coverage in the overlapping target age range (35-54 years): 80% after 1996, 82% before 1996. [‡]Weighted average of compliance with follow-up in 4 years from Table 2-3. [∫]Calculated as (total yearly number of smears – number of smears needed yearly to reach the observed coverage) × 1,000 / number of women in the target group. The number of smears needed yearly to reach the observed coverage equals (the population at risk in the target age range × observed coverage) / recommended interval.

Table 2-7. Comparison of the current Dutch cervical cancer screening programme with other cervical cancer screening programmes in terms of process indicators.[70, 135-140]

	The Netherlands	**Finland**	**England (UK)**	**Sweden**
Cervical cancer mortality per 100,000[†]	1.9	0.9	2.1	1.7
Organised programme characteristics				
Target age group	30-60	30-60	25-64	20-60
Screening interval (years)	5	5	3-5	2-4
Number of recommended smears per lifetime	7	7	12	14
Organised programme performance				
Share of smears within the programme[‡]	67%	37%	75%	39%
Target population / total number of smears	4.7	2.1	3.5	2.5
5-year coverage in the target group	77%	93%	81%	<82%[∫]
% Inadequate quality	1.0%	0.01%	9.3%	n.a.
% Positive smears[§]	2.3%	7.3%	6.4%	1.5%
- Of which highly positive[∞]	0.5%	0.6%	1.1%	n.a.

n.a.=not available. [†]Age-standardised rates, for the last available year: The Netherlands 2000, England and Finland 2002, Sweden 2001. [‡]The Netherlands: primary programme smears compared with total number of smears; Finland: programme smears compared with spontaneous smears; England and Sweden: programme smears compared with the total number of smears. [∫]3-yearly coverage. [§]Positive smears: the Netherlands, Finland: ≥Pap 2; England: ≥borderline dyskaryosis; Sweden: ≥CIN 1. [∞]Highly positive smears: the Netherlands: Pap3a2+; Finland: Pap3+; England: ≥moderate dyskaryosis.
Population data sources: The Netherlands: Central Bureau of Statistics;[23] Finland: Statfin; England: Department of Health;[137] Sweden: Swedish National Board of Health and Welfare.

(Appendix, Chapter 2.6.) were developed by recognized organisations of those professionals who perform smear-taking and pathology evaluation, and were based on available evidence. In many instances, such as for follow-up, they simplified and made clearer the recommended algorithms of care. In turn, adherence to the new protocols and guidelines was stimulated with those instruments that have proved to be effective in prevention, e.g. educational outreach visits and specialised software modules.[124,

[145] Responsibility for implementing the programme according to the agreed protocols, which was also secured with financial stipulations, was put in the hands of the regional screening centres that are backed by the covenant of the five professional groups with an interest in cervical cancer prevention. Early encouraging evidence from the new programme[62] could have further helped stimulate adherence to the pace set out in the protocols.

In conclusion, the 1996 evidence-based change of protocols and guidelines regarding the screening scheme, follow-up, administration and financing has brought about a considerable improvement in the cervical cancer screening programme in the Netherlands. It was made possible by continuous evaluation based on national pathology registry data, timely dissemination of results, and carefully developed change and implementation phases.

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2.6. Appendix: Overview of the changes in the cervical cancer screening protocols and guidelines in the Netherlands in 1996.

Table 2-A1. Protocols and guidelines regarding screening, financing and administration in the cervical cancer screening programme in the Netherlands, before and after 1996.

	Before 1996	After 1996
Screening scheme		
Target age range	35-53 years, but in practice it varied from 25-55	Uniformly 30-60 years
Screening interval	3 years	5 years
# Smears per lifetime	7	7
Invitation	Personal letter of the local health authority or the chosen GP, or through mass media	Personal letter of the local health authority or the chosen GP
Reminder	Not systematic	Mandatory
Follow-up	See Table 2-A2	See Table 2-A2
Financing		
Financing of the implementation of the programme	Non-earmarked municipal funds for health care in general	Earmarked funds managed centrally
Programme smear reimbursement (GPs and pathologists)	Health insurance, out-of-pocket payments, non-earmarked municipal funds	Earmarked funds managed centrally
Non-programme smear reimbursement	Reimbursed by health insurance, out-of-pocket payments	Not reimbursed and discouraged in guidelines for GPs (unless with medical complaints)
Administration		
Geographical coverage	85% of the municipalities (estimated)	National coverage
Coordination	Local, varies by region/municipality (local cancer registries, health authorities, municipalities, special work groups, none)	National (National Health Insurance Council. From 2006: National Institute for Public Health and the Environment)
Implementation	Local, varies by region/municipality (municipalities, local health authorities)	Local (screening centres), always under a covenant of the five local parties: health authority, cancer registry, GPs, pathologists, and gynaecologists
Programme evaluation	Not systematic	Integrated into the programme (with a special budget)

Table 2-A2. Guidelines regarding follow-up in the cervical cancer screening programme in the Netherlands, before and after 1996.

Pap class	Bethesda 2001 classification	Follow-up advice before 1996	Follow-up advice after 1996	Description after 1996
Pap 0	Unsatisfactory for evaluation	Repeat smear at 6 weeks	Repeat smear at 6 weeks	Inadequate sample
Pap 1	Negative for intraepithelial lesion or malignancy/Atrophy	Next screening round (3 years)	Next screening round (5 years)	No abnormalities
Ecc-[†]		Repeat smear at 12 months		No abnormalities
Pap 2 (borderline dyskaryosis)[‡,∫]	ASCUS/ASC-H/AGC	FU smear once every 12 months until a change in cytology	FU smear at 6 and 18 months	Abnormal squamous epithelial cells, atypical squamous metaplasia, atypical repair reaction of the endometrium, some atypical endocervical cells
Pap 3a1 (mild dyskaryosis)[‡]	ASC-H/LSIL/AGC favour neoplastic	FU smear at 3, 6 and 18 months		Mild dyskaryosis of the squamous epithelium, mildly or moderately atypical endocervical epithelium
Pap 3a2 (moderate dyskaryosis)	HSIL/AGC favour neoplastic	FU smear at 3, 6 and 18 months	Immediate referral	Moderate dyskaryosis of the squamous epithelium, mildly or moderately atypical endometrium
≥ Pap 3b (≥severe dyskaryosis)	HSIL/Squamous cell carcinoma/ AGC favour neoplastic/AIS/ Adenocarcinoma	Immediate referral		At least severe dyskaryosis of the squamous epithelium, at least severly atypical endometrium, at least severely atypical endocervical epithelium

FU=follow-up. [†]Negative smears without endocervical cells. Recommendation for a repeat smear at 12 months withdrawn in January 2002. [‡]BMD smears (Pap 2 + Pap 3a1). [∫]In 1996, the classification criteria for Pap 2 became more stringent in that the sole inflammation of epithelium and/or presence of specific microorganisms are to be classified under Pap 1.

CHAPTER 3. No Increased Risk for Cervical Cancer After a Broader Definition of a Negative Pap Smear

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3.1. Abstract

The definition of minimal relevant Pap smear abnormality is crucial for balancing the beneficial effects of screening (prevented mortality) with negative side-effects (the high positivity rate). After inflammation ceased to be defined as a borderline abnormal smear outcome in the Netherlands in 1996, the proportion of these smears dropped from 10% to less than 2%. Because this may have caused a loss in smear sensitivity, we analysed the changes in the incidence of cervical cancer after a negative Pap smear. All negative smears made at ages 30-64 in 1990-1995 (n=1,546,252) and 1998-2006 (n=3,552,716), registered in the national registry of histo- and cytopathology (PALGA), were followed for up to 9 years. During follow-up of the 1990-1995 smears, 377 women developed cervical cancer within 5,232,959 woman-years at risk, while during the follow-up of the 1998-2006 smears, 619 women developed cervical cancer within 11,210,675 woman-years at risk. The cumulative incidence after the definition change was not significantly higher than before: e.g. at 6 years, the cumulative incidence for smears made in 1990-1995 was 46 per 100,000 (95% CI: 41-52), and for smears in 1998-2006 was 48 per 100,000 (95% CI: 43-54), P=0.59. The hazard ratio for 1998-2006 compared to 1990-1995 adjusted for age, number of previous negative smears and history of abnormalities was 0.90 (95% CI: 0.78-1.03). In the Netherlands, a setting with high-quality cytological screening, treating smears with only signs of inflammation as negative leads to a considerably lower positivity rate without increasing the risk for cervical cancer after a negative smear.

3.2. Introduction

The number of women who benefit from cervical cancer screening (estimated at about 1%)[1, 146] is small compared to the number that bear a considerable burden of non-negative screening outcomes (2-10% per screening round).[147-151, Chapter 2] Women with non-negative cytologic findings are advised to stay in follow-up, and sometimes need to undergo colposcopy and treatment for preinvasive lesions which most often would not have developed into cancer.[32, 37] It is therefore crucial to set an appropriate threshold for the minimal level of relevant smear abnormality.

In the Netherlands, the current definition of this threshold, i.e. of borderline dyskaryosis (ASCUS), was agreed upon in 1996 as part of the major changes in the cervical cancer screening programme.[152, Chapter 2] Since 1996, inflammatory changes without dysplasia are seen as benign for cervical neoplasia and are included in the category of a negative rather than a borderline dyskaryotic Pap smear.[49] This guideline definition is comparable to that in many other countries, e.g. England and the USA.[40, 50, 153]

This change had a tremendous impact on the Dutch screening practice. During the 1980's and the early 1990's, about 10% of smears were judged borderline dyskaryotic (including inflammation).[51, 86] It was recommended to follow up this group of women with yearly smears until they had two consecutive negative smears and could thereafter rejoin the regular screening programme, or until their cytologic diagnosis worsened and they were referred to colposcopy.[154] In practice, it took several years before such a decision could be made.[Chapter 2] After the guidelines began to recommend classifying inflammation as a negative screening outcome, the proportion of borderline dyskaryotic smears decreased to less than 2% (1.5% for programme smears), whereas the remaining 80% of pre-1996 borderline smears are now classified as negative.[93]

Broadening the definition of a negative Pap smear with inflammation could cause a loss in smear sensitivity, though this has never been adequately studied. The Dutch observational experiment combined with the existence of nationwide comprehensive registration of total screening activity linked to diagnostic histological outcomes (including cancer) at the individual level since 1990 give a unique opportunity to study whether this has been the case. We analysed the changes in the incidence of invasive cancer after a negative smear (i.e., interval cancers) before and after the definition change.

3.3. Material and Methods

Information on all cervix uteri cytological and histological tests in the Netherlands registered until 31st March 2007 was retrieved from the nation-wide network and registry of histo- and cytopathology ("PALGA").[93] The registration began in the late 1970's, and achieved practically complete coverage of pathology registries in 1990.[81] PALGA identifies a woman through her birth date and the first four letters of the maiden name. This identification string enables the linkage of different tests belonging to

the same woman, and therefore also to follow individual testing histories (dates and diagnoses). The problem of false identity matches[127] was avoided by excluding women with 0.5% most common maiden names which corresponds to approximately 30% of all women.[76]

Registered screening histories were organized into screening episodes. An episode is defined as starting with a primary test (a smear or a biopsy) followed by secondary tests in case this test was abnormal (at least borderline dyskaryosis) or of inadequate quality. Follow-up or secondary tests were defined as the tests made within 4 years following the primary test, unless the follow-up of this primary smear had already been completed according to the guidelines[49, 59] (e.g., with two consecutive negative smears after a borderline dyskaryotic smear, or three consecutive negative smears after histologically confirmed cervical intraepithelial neoplasia (CIN)). All other tests were seen as primary tests.

We identified women with cervical cancer by selecting all PALGA records that included pathology codes for cervical cancer between 1994 and 2006. For these women, we reviewed the free text of all histology reports in PALGA. Complete follow-up (woman-years at risk and cases) was therefore left-censored at the beginning of 1994 and right-censored at the end of 2006. Cases and woman-years at risk were counted for at most 9 years from a negative primary smear until the primary smear of the next episode (or the date of the first histologically proven diagnosis of cervical cancer if it was diagnosed in the next episode (cases)), or else until 31st December 2006. Because during the analysed period the reason for smear-taking was not always registered in PALGA, all cancers originating in the cervix were counted as cases regardless of the reason for the primary investigation. Thus, all invasive cervical cancers diagnosed subsequent to a negative primary smear, including screen-detected, were counted as cases.

We compared negative primary smears taken in calendar periods 1990-1995 and 1998-2006. Smears made in 1996-1997 were excluded from the analysis because this was a transition period in which the proportion of borderline smears was still high but decreasing (1996: 5.5%, 1997: 3.3%).[93] First, the cumulative incidence rate (CIR) of interval cancer was calculated for each period for women aged 30-64 at the time of the primary smear. We focused on the 6-year CIR because this period covers the next screening round scheduled to take place 5 years after a negative smear. The 95% confidence intervals were estimated by non-parametric Kaplan-Meier product-limit estimator for log(hazard).[155] Second, the relative hazards were estimated by univariate and multivariate Cox regression with left and right censoring. In the univariate model, period (1998-2006 vs. 1990-1995) was entered as the explanatory variable; in the multivariate model also age at primary smear (grouped as 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60-64 years), the number of previous negative primary smears (1st negative smear vs. 2nd or later consecutive negative smear), and the history of abnormalities (no cytologic or histologic abnormality vs. at least a borderline negative smear in the past) were entered. Time dependency of the relative hazards was statistically tested by splitting the total follow-up time in two periods with a roughly equal number of cases. This leads to two new datasets where the full model is fitted on again. If the sum of the deviance of both sub-models is significantly lower than the deviance of the original model, the parameter estimates differ significantly between the two periods.

The detection rates of CIN grades 1, 2 and 3, and invasive cancer, i.e. the proportion of primary smears with a histologically confirmed cervical lesion, were calculated as the number of these lesions (numerator) per 1,000 smears in previously unscreened women aged 30-64 (denominator). To allow for follow-up, the most severe histologically confirmed diagnosis within 27 months of an abnormal smear was used as the final diagnosis. The age-adjusted odds ratios for periods January 1998-September 2004 vs. January 1994-December 1995 were estimated with logistic regression.

3.4. Results

We identified 1,546,252 negative smears made in 1990-1995 that contributed woman-years in the analysis, and 3,552,716 negative smears in 1998-2006 (Table 3-1). These were made in 1,136,631 and 2,005,627 women, respectively (2,314,250 women in total). The age at which these smears were taken was somewhat higher in the 1998-2006 period. The negative smears from the 1990-1995 period were more likely 1st rather than 2nd or later consecutive negative smears, and had therefore fewer previous abnormalities than those from the 1998-2006 period. 377 women were diagnosed with an invasive cancer in 5,232,959 woman-years at risk following a negative smear made in the 1990-1995 period, and 619 women in 11,210,675 woman-years at risk following a negative smear made in the 1998-2006 period.

Table 3-1. The characteristics of negative primary smears, by period: 1990-1995 (1,136,631 women) and 1998-2006 (2,005,627 women).

	1990-1995	1998-2006
Number of negative primary smears	1,546,252	3,552,716
Age at negative primary smear:		
30-39 years	42%	36%
40-49 years	39%	34%
50-59 years	17%	25%
60-64 years	2%	5%
Length of follow-up:		
Less than 2 years	16%	34%
2-4 years	42%	28%
4-6 years	20%	31%
6 years or more	23%	7%
Smear history:		
1st negative smear	40%	21%
2nd or later negative smear	60%	79%
Previous abnormalities:		
No previous abnormality	82%	74%
Previous at least cytological borderline dyskaryosis	18%	26%

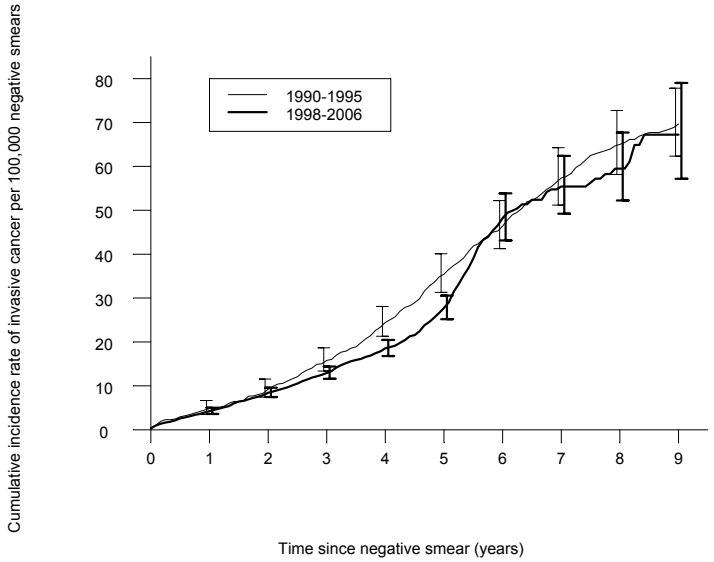

Figure 3-1. Incidence of cervical cancer: Cumulative incidence rate of invasive cancer per 100,000 negative primary smears at age 30-64, by calendar period in which the negative smear was taken.

The cumulative incidence rate (CIR) for the 1998-2006 negative smears was never significantly higher than that for the 1990-1995 smears (Figure 3-1). At 6 years, the CIR were 46 (95% CI: 41-52) and 48 (43-54) per 100,000 negative smears in the 1990-1995 and the 1998-2006 periods, respectively (P=0.59). The univariate hazard ratio was 0.88 (0.77-1.00) for 1998-2006 vs. 1990-1995. The multivariate hazard ratio adjusted for age at the primary smear, the number of previous consecutive negative smears, and the history of abnormalities was 0.90 (0.78-1.03) and therefore not substantially different from the univariate hazard. The test for time dependency of the relative hazards was statistically non-significant (P=0.45).

The detection rate of CIN 1 in previously unscreened women showed a 38% statistically significant decrease after 1998, whereas the detection rate of CIN 2+ increased by 22% (Table 3-2). The positive predictive value (PPV) of an abnormal primary smear (borderline dyskaryosis or worse) for CIN 2+ more than tripled in the recent period.

3.5. Discussion

In the Netherlands, treating inflammatory smears as a negative screening outcome decreased the proportion of borderline smears by 80%.[86] In spite of this, no increase in the risk for cervical cancer among women with a negative smear could be observed (Figure 3-1). This conclusion was based on a large observed series of cases that produced relatively narrow confidence intervals.

An increased cancer risk associated with inflammation could have been diluted in this comparison because it represents a small proportion (less than 10%) of all negative smears. In order to gain a more detailed insight into the risk associated specifically with inflammation, we additionally compared the detection rates of CIN and cancer before and after the change in the definition. We limited the analysis of the detection rates to previously unscreened women in order to obtain an unbiased comparison regarding their screening history. An unchanged detection rate would imply that there is no increased risk associated with inflammation, whereas a decrease would suggest an increased risk. The observed overall detection rates for CIN 2+, however, show that the programme's ability to detect cervical lesions was not diminished after 1996 (Table 3-2). Limiting these detection rates to the CIN lesions found after a borderline dyskaryotic smear also showed an increase (data not shown). The observed increase in the detection rates must be due to other factors. Indeed, since 1996 borderline smears had a more complete follow-up, and biopsies were taken more often than before 1996. [Chapter 2] On its own, the latter suggests that the narrower definition of a borderline dyskaryotic smear has been perceived as a more serious screening outcome, and tended to improve the quality of follow-up.

Since screen-detected cancers were included in the analysis, we evaluated whether our results were affected by a change (i.e., a decrease) in the screening frequency between the periods.[152] At 6 years, the 1998-2006 cumulative "incidence" of a subsequent smear after a negative smear was 9% lower than after a smear made in 1990-1995.[93] The effect of this small difference was further reduced because only part of the cancers (estimated <50%)[93] are screen-detected.

Table 3-2. Detection rates of CIN and invasive cancer per 1,000 primary smears in previously unscreened women aged 30-64, by calendar period, and age-adjusted odds ratios (95% CI).

	All primary smears			Abnormal primary smears†		
	1994-1995	1998-2004	OR	1994-1995	1998-2004	OR
Number of smears	150,704	387,331		18,665	16,594	
CIN 1	4.8	3.0	0.7 (0.6-0.8)	31	62	2.0 (1.8-2.2)
CIN 2	2.9	3.4	1.1 (0.9-1.2)	21	73	3.1 (2.7-3.5)
CIN 3	7.8	9.9	1.2 (1.1-1.2)	60	222	3.9 (3.6-4.1)
Cervical cancer	1.2	1.2	1.1 (0.9-1.3)	9	26	3.4 (2.8-4.0)

†Borderline dyskaryosis or worse.

All smears and histologically diagnosed CIN and cervical cancers are in principle registered in PALGA. In the Netherlands, this is the only comprehensive registry that links the cancer cases with their screening history. In PALGA, the total number of women aged 30-74 with an incident cervical cancer in the period 1994-2003 was 10% higher than published by the Cancer Registry.[4] There was no trend in the differences between the two sources by calendar year. Thus, while the interval cancer rates may have been somewhat overestimated, the comparison of the periods was not biased.

Even though the recommended definition of borderline dyskaryosis is similar in several countries, the observed smear abnormality rates vary substantially. The diagnosis of borderline dyskaryosis is made in 1.5% of the programme smears between ages 30 and 64 in the Netherlands, 3% in England and almost 5% in the USA.[93, 156, 157] This variation could be due to genuine differences in the background risk, or to the differences in smear interpretation. The differences in the observed incidence rates are small,[1] while the country-specific screening intensities are similar.[118, 150, Chapter 2] This suggests that the background risk is comparable. On the other hand, systematic differences in the interpretation of borderline dyskaryosis have been shown to exist, with the tendency in England and the USA to classify subtler lesions as borderline abnormal.[158] This suggests that there may still be room for decreasing the smear abnormality rates in these other countries.

This observational experiment with broadening the definition of a negative smear took place in a setting with high-quality cytology practice. In the Netherlands, strict quality assurance measures aimed at improving and standardising smear interpretation are set out in professional guidelines.[49, 58] Nation-wide pro-forma reporting for cytology was introduced in 1996, and instruction CD-ROMs with guidelines and visual analogues were distributed to every cytotechnician.[49, 159] Laboratory-specific feedback is provided to all pathology laboratories in the framework of continuous monitoring.

The balance between the positivity rate (i.e., the proportion of smears with a positive outcome) and increased cancer prevention is also relevant in HPV screening. The positivity rates of the most widely used HPV tests are 2 to 4 times higher than those of conventional cytology.[98, 99] Partly, the higher abnormality rate will represent a loss in specificity, but unlike with inflammation, a gain in sensitivity can be expected.[98, 99] These will have to be weighted against each other after pooling the data on interval cancers from the currently on-going HPV trials,[160] which will allow an even more accurate estimate of the true increase in the sensitivity.

In conclusion, excluding inflammatory changes from the definition of borderline dyskaryosis in the Netherlands led to an 80% decrease in borderline abnormal smears without increasing the risk for cervical cancer after a negative smear.

Acknowledgments

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CHAPTER 4. Cervical Cancer Incidence After Several Negative Smears by Age 50

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Submitted.

4.1. Abstract

Objectives. After several consecutive negative Pap smears by age 50, the detection rates of preinvasive cervical lesions (CIN) are considerably lower than among similarly screened younger women. Several authors concluded that continued screening in these older women might be inefficient. Because CIN is only a surrogate outcome measure, and its regression rates are age-dependent, we compared the incidence of cervical cancer after several negative smears at different ages. *Design.* Prospective observational study of cervical cancer incidence after the third consecutive negative smear based on the individual-level data in a national registry of histo- and cytopathology (PALGA). *Setting.* The Netherlands, national data. *Population.* 218,847 women aged 45-54, and 445,382 aged 30-44 years at the time of the third negative smear. *Main outcome measures.* The 10-year cumulative incidence of interval cervical cancer (CIR). *Results.* 105 women developed cervical cancer within 2,595,964 woman-years at risk after the third negative smear at age 30-44, and 42 within 1,278,532 woman-years at risk after age 45-54. During follow-up, both age groups had similar levels of screening. After 10 years of follow-up, the CIR of cervical cancer was similar: 41 per 100,000 (95% CI: 33-51) in the younger, and 36 per 100,000 (24-52) in the older group (P=0.48). The CIR of CIN 1+ was twice as high in the younger compared to the older group (P<0.001). *Conclusions.* The risk for cervical cancer after several negative smears by age 50 is similar to that at younger ages. It would thus not be consistent to stop screening women with several consecutive negative smears after age 50, while not relaxing the screening policy at younger age.

4.2. Introduction

The debate on earlier cessation of cervical cancer screening for women with several consecutive negative smears by age 50 has been on-going for about 15 years without drawing clear conclusions in terms of guideline recommendations. Several authors studied this issue by analysing the detection rates of preinvasive cervical lesions (CIN) in these women.[18, 90-92, 95-97, 161, 162] In general, they observed considerably lower detection rates than in similarly screened younger women. Based on this finding, they argued that continued screening is not as efficient as among younger women, and could be stopped at the expense of only a limited increase in the incidence of cervical cancer among these older women.[90, 92, 95, 97] If true, this could result in considerable savings for the screening programmes. For example, in the Netherlands it would apply to about half of the women attending screening around age 50.[93]

However, because there is strong evidence that CIN lesions have a higher probability to progress to invasive cancer at older ages,[34] lower detection rate of CIN after age 50 alone do not represent conclusive evidence for lower screening efficiency. This is reflected in the fact that the guidelines have not been adjusted. Data on invasive cancer has since become available in a Dutch nationwide pathology registry with screening histories linked to diagnostic histological outcomes (including cancer) at the individual level. The aim of this paper was to measure the incidence of invasive cancer after several consecutive negative smears in women around age 50, and in younger women. This will bypass the problems associated with using CIN lesions, and enable a more conclusive evaluation of whether there is more reason to relax screening in older than in younger women with similar negative screening histories.

4.3. Methods

Data

From the Dutch nationwide network and registry of histo- and cytopathology (PALGA), we retrieved information on all cervix uteri cytological and histological tests until 31st March 2004. The registration began in the late 1970's, and achieved practically complete coverage of pathology laboratories in 1990.[81] In the Netherlands, cervical cancer screening became widespread after an extensive pilot project that started in 1976. Around 1980, the program inviting women aged 35-53 with a 3-year interval reached an almost national coverage. However, much opportunistic screening in young women coexisted next to the organized program.[163] In 1996, the program was reorganized. Women are since invited once every 5 years between ages 30 and 60.[56] In 2003, 77% of women at risk (=with a cervix) in this age group had at least one smear in the preceding 5 years.[Chapter 2] The most commonly used screening tool is a conventional Pap smear, although the share of liquid-based cytology smears is increasing.

In PALGA, women are identified through their birth date and the first four letters of the (maiden) family name. This identification code enabled linkage of the tests belonging to the same woman, allowing following the individual screening and disease histories. Because this code is not always unique,[127] we excluded women with 0.5% of the most common first four letters

of the family name, i.e. about 30% of women.[76] We identified cervical cancer cases by (manually) checking the free text of the pathology reports for all excerpts that included pathology codes for cervical cancer. This was done for the period 1994-2002. The follow-up (person-years at risk and cases) in the present analysis was therefore left-censored at the beginning of 1994 and right-censored at the end of 2002.

Statistical analysis

We selected women in two age groups, 45-54 ("the older group") and 30-44 ("the younger group"), if they had the third consecutive negative primary smear in this age interval at any time since the beginning of the registration. Women with prior histological (CIN 1+) or cytological (borderline dyskaryosis or worse) abnormalities were excluded. Women were followed-up from the date of the third negative smear until the date of the first diagnosis of cervical cancer, or until end of 2002. We could not censor the follow-up in case of death from other causes because no information on the time of death was available. However, we estimate that the potential impact on our results was small because the female mortality rate in the Netherlands below age 65 is low.[164]

For both age groups, we first calculated the cumulative incidence rate (CIR) of cervical cancer in the period 1994-2002 by time since the third negative smear. Because for the majority of women, about 85% in the older and 80% in the younger group, at most 10 years of follow-up was available, we focused on the CIR during these first 10 years. The difference in the CIR between the age groups was tested for statistical significance assuming a Poisson distribution for the number of women with cancer, i.e. cases (H_0: no difference in the CIR between the age groups). We estimated the 95% confidence intervals using the non-parametric Kaplan-Meier product-limit estimator for log(hazard).[155] Second, the difference in the incidence rates between the two age groups during the whole period in follow-up (the hazard rate) was tested by Cox regression with left and right censoring. Time dependency of relative hazards was statistically tested by splitting the total follow-up time in two periods with a roughly equal number of cases.

4.4. Results

We identified 219 thousand women in the older group and 445 thousand in the younger group that met our inclusion criteria (Table 4-1). The average interval between the 3 consecutive

Table 4-1. The description of the study population, by 5-year age groups.

	Age at entry				
	30-44 years			45-54 years	
	30-34 years	35-39 years	40-44 years	45-49 years	50-54 years
Number	126,748	156,435	162,199	124,254	94,593
CIR†	36 (23-58)	39 (26-59)	45 (32-61)	38 (22-66)	33 (21-53)

†Cumulative incidence rate per 100,000 women at 10 years after the third negative smear (95% CI).

Table 4-2. Incidence of invasive cervical cancer after the third consecutive negative smear for two age groups. Tabulated are woman-years,† number of invasive cancers, and the cumulative incidence rate of invasive cancer per 100,000 women (95% CI).

Time since the third negative smear	Age at entry						P‡
	30-44 years			45-54 years			
	Woman-years	Women with invasive cancer	Cumulative incidence rate (95% CI)	Woman-years	Women with invasive cancer	Cumulative incidence rate (95% CI)	
Third negative smear							
	324,512	4		172,920	3		
1 year			1 (0-3)			2 (1-5)	0.66
	628,471	16		344,825	16		
3 years			6 (4-10)			11 (7-17)	0.09
	563,725	27		304,194	5		
5 years			16 (12-21)			14 (10-21)	0.65
	837,359	43		378,075	13		
10 years			41 (33-51)			36 (24-52)	0.48
	200,225	11		65,373	4		
15 years			70 (51-95)			73 (39-135)	0.85
	41,672	4		13,145	1		
20 years			128 (79-207)			105 (50-219)	0.27
Total	2,595,964	105		1,278,532	42		

CIR: cumulative incidence rate per 100,000 women. †Accrued between 1st January 1994 and 31st December 2002. ‡Two-sided, for difference in the CIR between the two age groups at specific time points.

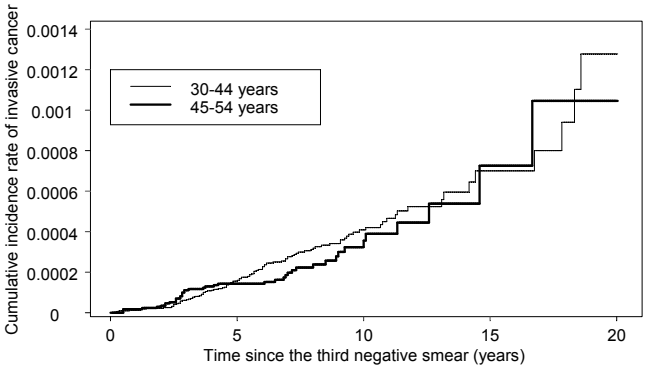

Figure 4-1. The cumulative incidence rate for invasive cancer, by age group and time since the third consecutive negative smear.

Table 4-3. Screening intensity: Number of primary screening tests after the third consecutive negative smear. Average number of years at risk for further smears after the third negative smear were 6.7 years and 6.4 years among women aged 30-44 and 45-45, respectively.

	Age at entry	
Number of primary screening tests after the third negative smear	**30-44 years**	**45-54 years**
0	35%	35%
1	27%	33%
2	18%	18%
3	10%	8%
≥4	10%	7%
Total	100%	100%

negative smears (i.e., between the first and the second, and the second and the third smear) was 40 months in the older group and 39 months in the younger group. In the period between 1st January 1994 and 31st December 2002, 1.3 and 2.6 million person-years in follow-up accrued in these women, respectively, an average of 5.84 and 5.83 years per woman (Table 4-2). The two groups had a similar rate of screening after the third negative smear (Table 4-3): about one third had none, about one third had one, and the remaining third had more than one further primary test registered. Forty-two women in the older, and 105 women in the younger group developed cervical cancer (Table 4-2).

During follow-up, the difference in the CIR between the age groups was never statistically significant (Figure 4-1, and Table 4-2). Pooling women into two large age groups does not seem to have affected this result, as the CIR at 10 years in smaller 5-year age groups also did not differ significantly (Table 4-1; P=0.24).

The overall hazard ratio was 0.84 (95% CI: 0.59-1.21) for the older compared with the younger group. The test for time dependency of the relative hazards was statistically non-significant (P=0.86).

We also calculated the CIR with CIN 1+ as the endpoint (Table 4-4, and Figure 4-2). By 10 years, the CIR was 12.6 (95% CI: 12.1-13.1) per 1,000 women in the younger group and 5.9 (95% CI: 5.5-6.5) in the older group. The difference between both groups was statistically significant throughout entire follow-up. Using CIN 2+ or CIN 3+ as the endpoint instead of CIN 1+ showed a similar relationship between the two age groups (data not shown).

4.5. Discussion

The risk for developing cervical cancer after the third consecutive negative smear among women around age 50 was practically the same as that among younger women (relative risk 16% lower, 95% CI: 41% lower to 21% higher). This outcome was not biased by differential screening during follow-up because there was no difference between the age groups in this respect (Table 4-3). Evidence available in the literature does not show that either the screening sensitivity for high-grade CIN or the effectiveness of screening-induced CIN treatment substantially decreases with age.[38, 45, 165-167] Therefore, it is reasonable to assume that after several consecutive negative smears the screening efficiency in terms of detection and prevention of cervical cancer is at the same level around age 50 as it is at younger ages.

We observed a lower risk for CIN 1+ in the older group (Table 4-4 and Figure 4-2). In this respect, our data is consistent with that of others.[90, 92, 95, 97] This corroborates that CIN is not an accurate intermediate endpoint for the question addressed.

Because women were included as soon as they had the third consecutive negative smear, younger women will on average have been screened more intensely than older women; the latter may therefore be at higher risk. However, the selection criterion of being disease-free on 3 consecutive screenings, and the finding that the screening attendance after the third negative smear was similar in both groups, make such a bias unlikely. We tested this in an additional analysis, where we included women from the younger group also in the older group if they continued to have negative smears after age 44. The result was the same: the 10-year CIR in the older group in this case slightly decreased from 36 (95% CI: 24-52) to 34 (25-48) per 100,000.

The similarity in the CIR between the two age groups is not unexpected given the observed age-specific incidence before screening became widespread[2] (i.e. before ca. 1970 in most developed countries). In the Netherlands, as well as in several Western-European countries, the pre-screening incidence rose rapidly until a peak around ages 44 to 49 years, and declined thereafter. This would translate into roughly equal levels of CIR during the first 10 years for the two age groups. In some other countries, like the UK and the USA, the decline in the pre-screening incidence at older ages is slower. If this pattern is due to a truly different age effect and not to a cohort effect, the cancer incidence reduction gained through continued screening in the older group would be even relatively higher than in the Netherlands.

The question of age-specific screening efficiency can be further explored by comparing the average number of life-years lost in absence of screening. Younger women have a longer remaining life expectancy than older women, but they also have lower

Table 4-4. Incidence of CIN after a third consecutive negative smear for two age groups. Tabulated are woman-years,† number of CIN 1+, a cumulative incidence rate of CIN 1+ per 100,000 women (95% CI).

	Age at entry						
	30-44 years			45-54 years			P‡
Time since the third negative smear	Woman-years	Women with CIN 1+	Cumulative incidence rate (95% CI)	Woman-years	Women with CIN 1+	Cumulative incidence rate (95% CI)	
Third negative smear							
	324,381	233		172,850	90		
1 year			72 (63-82)			52 (42-64)	0.008
	627,524	584		344,441	172		
3 years			258 (241-277)			152 (135-172)	<0.001
	561,412	834		303,363	240		
5 years			555 (529-583)			310 (284-339)	<0.001
	829,336	1,192		375,786	224		
10 years			1,258 (1,209-1,308)			594 (547-645)	<0.001
	196,753	197		64,635	30		
15 years			1,707 (1,622-1,796)			769 (686-862)	<0.001
	40,898	24		12,995	5		
20 years			1,986 (1,841-2,143)			920 (772-1,096)	<0.001
Total	2,580,304	3,064		1,274,070	761		

†Accrued between 1st January 1994 and 31st December 2002. ‡Two-sided, for difference in the CIR between the two age groups at specific time points.

lethality rates from cervical cancer. The remaining female life expectancy in the Netherlands is 42, 33 and 24 years at ages 40, 50 and 60, respectively,[168] while the 5-year mortality rate from clinical cervical cancer increases from 50% to 70% and 75%, respectively, at the same ages.[169] Assuming that the 5-year mortality rates approximate the total lethality, around 20 years are lost per incident case for all 3 ages, which means that decreasing life expectancy and the increasing cancer lethality compensate each other. At even older ages, however, the number of life-years lost per incident case starts to decrease.

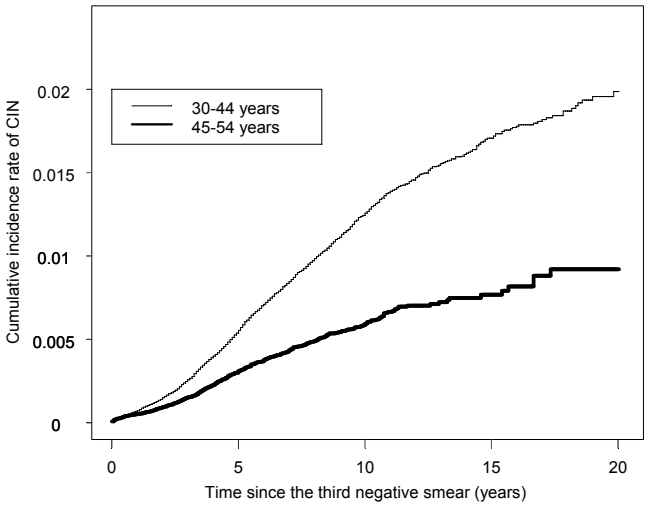

Figure 4-2. The cumulative incidence rate for CIN 1+, by age group and time since the third consecutive negative smear.

Our data does not permit a simple extension of our study to older ages. For example, in 79,586 women satisfying the criteria at ages 55 to 64 years, the 10-year CIR was 47 per 100,000 (95% CI: 23-99), and was statistically comparable to that in women below age 55. However, women aged 55-64 years had a considerably lower screening intensity after the third negative smear: 60% had no further smear compared with 35% in women below age 55. In women above 64, screening intensity decreases even further. This diminishes the actual comparability of women aged 55 or older with women below that age, and as a consequence, a clear conclusion on the relative screening efficiency cannot be drawn.

We selected women with negative screening histories, i.e. women who never had cytological or histological evidence of neoplasia. In everyday practice, though, complete screening histories may not always be known and may contain abnormalities. Women with prior abnormalities, i.e. at least abnormal cytology, remain at higher risk for invasive cancer despite later consecutive negative smears.[170] In our data, inclusion of women with screen-detected abnormalities followed by 3 consecutive primary negative smears does not affect the two age groups differently: the CIR at 10 years is 42 (95% CI: 30-57) per 100,000 women in the older and 42 (34-51) in the younger group.

The continued risk for cervical cancer is consistent with the considerable incidence of HPV infections in older

women.[171, 172] When primary screening would be done by HPV testing, our conclusions would therefore remain the same. This would also mean that the HPV vaccine may only succeed in attaining its full potential of eradicating up to 70% of cervical cancer if it offers protection from a persistent HPV infection for many decades, i.e. also after age 50. This again will depend strongly on the – unknown – proportion of infections around and after age 50 that are due to reactivated latent infections acquired earlier in life.[173, 174]

By being able to use invasive cancer as the relevant endpoint, our analysis gives a more evidence-based answer to the on-going discussion on continued screening in women with several negative smears by age 50. It showed that it would not be consistent to stop screening in these women while not relaxing the screening policy for women with similar screening histories at younger age. In this respect, our conclusion lends support to the current cervical cancer screening guidelines in England and other developed countries,[111, 175-178] which do not discriminate women by their age up to 60 to 65 years.

Whether individual tailoring of recommendations for further screening by using the information on individual screening histories would be an efficient and feasible alternative in any age group to the current fixed schedule, remains to be explored.

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CHAPTER 5. Joint European Study on the Long-Term Predictive Values of Cytology and Human Papillomavirus testing in Cervical Cancer Screening

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5.1. Abstract

Objective. We aimed to obtain large-scale and generalizable data on the long-term predictive values of cytology and Human Papillomavirus testing for development of cervical intraepithelial neoplasia grade 3 or cancer. *Design*. Multinational cohort study with joint database analysis. *Setting*. Seven primary Human Papillomavirus screening studies in 6 European countries. *Participants*. 24,295 women attending cervical screening who were enrolled into Human Papillomavirus screening trials and had at least 1 cervical cytology or histopathology during follow-up. *Main outcome measure*. Long-term cumulative incidence rate of cervical intraepithelial neoplasia grade 3 or cancer. *Results*. The cumulative incidence rate of cervical intraepithelial neoplasia grade 3 or cancer after 6 years was considerably lower among Human Papillomavirus-negative women (0.27% (95% CI: 0.12-0.45%)) than among cytology-negative women (0.97% (95% CI: 0.53-1.34%)). By comparison, the cumulative incidence rate of cervical intraepithelial neoplasia grade 3 or cancer for cytology-negative women at the most commonly recommended screening interval in Europe (3 years) was 0.51% (95% CI: 0.23-0.77%)). The cumulative incidence rate of cervical intraepithelial neoplasia grade 3 or cancer among cytology-negative/Human Papillomavirus-positive women increased continuously over time, reaching 10% at 6 years, whereas the cumulative incidence rate of cervical intraepithelial neoplasia grade 3 or cancer among cytology-positive/Human Papillomavirus-negative women remained below 3%. *Conclusions*. A consistently low 6-year cumulative incidence rate of cervical intraepithelial neoplasia grade 3 or cancer among Human Papillomavirus-negative women suggests safety of cervical screening strategies with Human Papillomavirus testing at 6-yearly intervals.

5.2. Introduction

Cytological screening has reduced cervical cancer incidence in countries with organized screening[179] but there were still an estimated 68,000 incident cases in Europe in 1995.[180] Cytology has limited reproducibility[181] and both meta-analyses and pooled analyses of cross-sectional studies have established that Human Papillomavirus (HPV) tests have higher sensitivity than cytology in detecting high-grade cervical intraepithelial lesions (CIN)[46, 48] and that combined HPV and cytology testing has very high negative predictive values for CIN.[182-184] However, cost-effectiveness modeling of screening strategies is highly dependent on reliable and generalizable estimates of the longitudinal, long-term predictive values of testing. The long-term negative predictive value (NPV) is the main determinant of the safe screening interval to use, a key factor for the cost-efficiency of a screening program. The long-term positive predictive value (PPV) is an important measure of the extent of unnecessary procedures induced by screening, another major factor in cost-efficiency evaluations. As low and moderate grades of CIN often regress, predictive values to be used for modeling should ideally use CIN grade 3 or cancer (CIN3+) as the outcome.[12]

Several randomized controlled trials are currently being conducted to compare HPV-based primary screening with conventional cytology screening.[98, 99, 185-189] Data from these trials

indicate that HPV-based screening results in detection of more high-grade CIN lesions (a higher sensitivity) but a reduced specificity compared to cytology-based screening. The randomized trials found that the increased sensitivity for CIN3+ is not merely overdiagnosis as there is a correspondingly lower CIN3+ incidence in the future,[98, 99, 185, 186] further establishing the validity of using CIN3+ as endpoint in studies of HPV-based cervical screening.

However, most of the cohort studies and the randomized trials have observed only limited numbers of CIN 3+ cases on longer term follow-up, resulting in limited statistical power for estimating the critical factor for deciding the appropriate screening interval: the CIN3+ rate among screen-negative women. Furthermore, clinical and diagnostic practices vary between European countries and different studies have often used different methods for evaluation, making meta-analyses difficult.

To obtain large-scale and generalizable data on long-term CIN3+ predictive values, seven HPV screening studies in six EU countries, each investigating the predictive value of primary HPV screening for future CIN3+, supplied primary data to a common database for joint statistical analysis. Variability between studies was assessed and the overall long-term predictive values for CIN3+ estimated.

5.3. Material and Methods

The seven prospective HPV studies in six European countries that supplied the data to the common database for joint statistical analysis were designed to evaluate primary cervical screening using HPV testing. The design of the seven studies, inclusion and exclusion criteria, setting and location is summarized in Table 5-1. All studies used routine cytology as currently practised in their country. The different HPV tests used are listed in Table 5-1 and further described for each country below. For all studies the persons executing either test were unaware of the results of the other test. Comparability and reproducibility of the 2 major HPV tests used (Hybrid Capture 2 and GP5+/6+ polymerase chain reaction (PCR)) was evaluated using kappa statistics.[190] All studies were approved by the Ethical Review Boards in their respective countries. Recruitment was consecutive and data collection prospectively planned.

Denmark

Women in the general population were enrolled in a prospective cohort study of the natural history of HPV and cervical neoplasia between 1993-95. They were interviewed and a cervical smear for cytology and cervical swabs for HPV DNA detection using Hybrid Capture 2 (HC 2) were taken.[191] In Denmark, every citizen has a unique 10-digit personal identification number that was linked to the national Pathology Data Bank (a nationwide computerized pathology register containing all cytological and histological diagnoses in Denmark) to allow follow-up. Data from women with double negative tests were supplied to the joint database.

Germany – Hannover and Tuebingen studies

In 1999-2000 women 30 years or older were invited to the medical universities in Hannover or Tuebingen for a prospective cohort study (HAT-trial) on HPV screening among women 30 years or older.[183] In Hannover women were followed with colposcopy every 6-12 months if they had a positive HC 2 test or positive cytology at baseline. Double negative women were followed with annual Pap smears and in addition, 5 % of the women with a double negative test result were referred for colposcopy five years later. In Tuebingen, patients with a double negative result at baseline were followed up with cytology and HC 2 test after 5 years and if either test was positive they were referred for colposcopy.

United Kingdom

The UK study was based on women attending routine screening in West London to evaluate HPV-based screening in women aged 35 or over during 1994-1997.[16] DNA analysis was initially performed using the PCR/SHARP system detecting HPV types 16, 18, 31, 33, 54, 51, 52, 56 and 58 and women with a positive HPV test or abnormal cytology were referred for colposcopy. The samples were retrospectively analysed using HC 1 for the first half of the study and by HC 2 for the second half of the study. HPV results reported in our analyses are based on Hybrid Capture results. All women were followed-up using the National Health Service computerised call/recall system which records all smears and their results. In addition, all women were invited to and 520 women attended a follow-up visit with HPV test and colposcopy.

France

Women who participated in biennial or triennial routine screening from 1997 to 2002 in Reims, France, were invited to participate in a study to evaluate HPV testing (HC 2) in cervical cancer screening.[192] All women with abnormal cytology were referred for colposcopy. Women with a positive HPV test but normal cytology were recalled after 6-12 months for a repeat cytological smear and HPV test. If a cytological abnormality was found or if the women had a persistent HPV infection she was referred for colposcopy. Women with normal cytology and a negative HPV test at baseline were followed with standard biennial or triennial cervical screening. A random 15% of baseline double negative women were also referred for colposcopy.

Sweden

Between 1997 and 2000, women aged 32-38 years who took part in organized cervical screening in five regions of Sweden (Gothenburg, Malmö, Stockholm, Umeå and Uppsala) were invited to participate in a randomized population-based trial of primary HPV screening using general primer GP5+/6+ PCR.[193] In the intervention arm, HPV positive women were invited for a second cytology and HPV test at least a year later, together with a similar number of women randomly selected from the control arm. Women with persistent HPV infection, as well as a similar number of women from the control arm, were invited for colposcopy.[193] Women with abnormal cytology were referred for colposcopy in accordance with established clinical algorithms. All women in the intervention arm and the randomly selected women from the control arm are included in this paper. All study participants were followed by registry linkages with comprehensive regional cytology and pathology registries using unique personal identification numbers.

Spain

During 1997 to 2001,[194] women were enrolled who were either randomly selected from the general population of the Barcelona metropolitan area or were attending 9 family planning clinics

Table 5-1. Study Characteristics of seven European HPV screening studies.

Study	Numbers initially screened	Numbers analysed[†]	Age	Entry Criteria	HPV test	Follow-up[‡]	Histology
Germany-Hannover	4,699	4,107	≥30	No history of abnormal smear, CIN, or treatment for cervical disease in the past year and not pregnant.	HC 2	If cyt+ or HPV+ immediate and annual colposcopy[ʃ] for 5 years. 5 % of cyt-/HPV- to colposcopy after 5 years.	Blinded central review
Germany-Tuebingen	672	670	≥30	No history of abnormal smear, CIN, or treatment for cervical disease in the past year and not pregnant.	HC 2	Cyt-/HPV- to new tests after 5 years and if either positive referred to colposcopy.	Blinded central review
Sweden	6,448	5,671	32-38	Participating in organized screening.	GP5+/6+ PCR	Cyt-/HPV+ were invited for new test >1 year later, if persistent HPV+ referred to colposcopy. Similar number of women randomly referred to colposcopy. Database linked with Regional Pathology registries.	Regional pathology laboratories[§]
Denmark	2,287	2,274	20-29	No history or current evidence of cervical neoplasia.	HC 2	Study data base linked with the National Pathology Registry.	Regional pathology laoratories[§]
UK	2,720	2,322	≥35	No previous cervical treatment or abnormal smear within the last 3 years	SHARP-PCR, HC 1 and HC 2	SHARP-PCR+ or cyt+ referred to colposcopy.	Blinded central review
France	17,247	7,935	No age limits	No abnormal smear or untreated cervical lesion in the past 2 years. Not HIV positive.	HC 2	If cyt-/HPV+ new tests after 6-12 months and if persistent HPV+ referred to colposcopy. 15 % cyt-/HPV- referred to colposcopy.	Blinded central review
Spain	2,012	1,316	Matched to general popu-lation	Population registry or attending screening	HC2	Follow-up tests 1 and 5 years later. Persistently HPV-positive women referred to colposcopy.	Regional pathology laboratories[§]

[†]Women with at least one follow-up cytology or histology. [‡]Follow-up procedures performed in addition to the routine clinical practice. [ʃ]Assessment by colposcopy. [§]Blinded to HPV status.

for routine screening. Both enrolment strategies performed frequency matching to the underlying general population. The aims of the study were to estimate the incidence and prevalence of genital HPV infection and to evaluate the predictive value of cytology and HPV testing for future CIN at 1 and 5 years follow-up. HPV testing was done by means of HC2. Women from the general population were referred for colposcopy if there was an abnormal cervical cytology or if there was a persistent HPV positive test at the end of follow-up. The women from the family planning clinics were referred for colposcopy if there was an abnormal cervical cytology or if there were two consecutively positive HPV tests.

Statistical analysis

From the joint cohort, only women with adequate cytology and HPV test at baseline, and with at least one follow-up cytological or histological test were included in the present analysis. In the analysis of the joint cohort, abnormal cytology was regarded as the equivalent of atypical squamous cells of uncertain significance (ASCUS) or worse for all participating studies. Women were followed from the date of the baseline test. Incidence is dependent on the number of person-years of follow-up time and for a screen-detectable disease follow-up requires having attended screening. Therefore, follow-up was censored at the date the CIN3+ lesion (CIN 3 or invasive cancer, including squamous and adenocarcinoma) was diagnosed, or at the last registered testing date.

First, the country-specific estimates of the cumulative incidence rate (CIR) of CIN 3+ by original baseline group (cyt-/HPV-, cyt-/HPV+, cyt+/HPV- and cyt+/HPV+) were estimated; their 95% confidence intervals (CI) were estimated using the non-parametric Kaplan-Meier product-limit estimator for log(hazard).[155] Second, whether the inhomogeneity between the different studies in the joint cohort is a major factor influencing results can be tested for by comparative analysis of systematically drawn subsamples of the joint cohort, so-called bootstrap analysis.[195] The bootstrap stratified random subsample was constructed by drawing, with replacement, first from studies and then from individuals within studies. 1,000 bootstrap replicas were constructed and analysed analogous to the original country-specific analysis. The mean of these 1,000 replicas was used as the pooled estimate of the CIR corrected for heterogeneity, and 2.5 and 97.5 percentiles as estimates for the 95% CI. As a measure for heterogeneity, the original cohort-specific 95% confidence intervals were compared with those obtained by the multilevel bootstrap. This can be transformed into an estimate of the over-dispersion parameter (or "scale" parameter), where the value 1.00 points to no heterogeneity among the studies and values >1.00 point to increasing levels of heterogeneity.[196, 197] Third, we calculated the test performance indices for cytology alone, HPV test alone and cytology and HPV test combined (at least one of the two positive). Because data was not complete for all 4 original baseline groups, studies from Denmark and Tuebingen were excluded from these analyses. These indices were calculated using 2×2 tables based on the CIR at 72 months for the different baseline test combinations, weighted by the proportion (adjusted for heterogeneity) of each of these subgroups at baseline.[198] The 95% confidence intervals around the indices were obtained by bootstrap.[196] All analyses used S-PLUS 6.0 Professional Release 1.

Role of the funding sources

None of the funding sources had any involvement in study design; in the collection, analysis, and interpretation of data; in the writing of the report or in the decision to submit the paper for publication.

5.4. Results

Out of 24,295 women included in the pooled analyses, 381 developed histologically confirmed CIN3+ during 6 years of follow-up (Table 5-2). The positive predictive value for future CIN3+ was highest among women with baseline abnormal cytology and positive HPV test (cyt+/HPV+) (CIR: 34 %, 95% CI: 26.8-45.4%) (Figure 5-1). Women with normal cytology but positive HPV test (cyt-/HPV+) had a continuously increasing CIR of CIN3+, eventually reaching 10 % (95% CI: 6.2-15.1%) after 6 years. Women with abnormal cytology and negative HPV test (cyt+/HPV-) had a CIR for CIN3+ of 2.7 % (95% CI: 0.6-6.0%). Women with both normal cytology and negative HPV test (cyt-/HPV-) had a very low risk of future CIN3+ (CIR: 0.28 %, 95% CI: 0.10-0.47%). The CIR of CIN3+ after being cyt-/HPV- was compared to the CIR of CIN3+ for normal cytology alone and negative HPV test alone (Figure 5-2). At 6 years of follow-up, the CIR of CIN3+ was significantly lower among HPV-negative women (0.27% (95% CI: 0.12-0.45%) than among cytology-negative women (0.97% (95% CI: 0.53-1.34%). By comparison, the CIR of CIN3+ at the most commonly recommended screening interval in Europe (3 years) was 0.51% (95% CI: 0.23-0.77%) for cytology-negative women and 0.12% (95% CI: 0.03-0.24%) for HPV-negative women. At 5 and 4 years of follow-up, the CIRs were 0.25% (95% CI: 0.12-0.41%) and 0.19% (95% CI: 0.08-0.32%) for HPV-negative women compared to 0.83% (95% CI: 0.50-1.13%) and 0.69% (95% CI: 0.39-0.98%) for cytology-negative women. There was very little difference in CIR for CIN3+ between double negative and HPV-negative women (Figure 5-2). The CIR for CIN3+ among HPV-positive women was lower than for women with abnormal cytology, but increased continuously and gradually approached the CIR of cytology-positive women (Figure 5-3).

Table 5-2. Number of women in the analysis, and the number of CIN 3+ diagnosed within 6 years of baseline.

Baseline group	Number of women at baseline†	Number of women still in follow-up after:		Number of women with CIN 3+
		60 months	72 months	
Cyt-/HPV-	21,060	7,019	4,571	32
Cyt-/HPV+	1,962	268	111	107
Cyt+/HPV-	436	89	46	10
Cyt+/HPV+	837	67	40	232
Total	24,295	7,443	4,778	381

†With at least one cytology or histology follow-up.

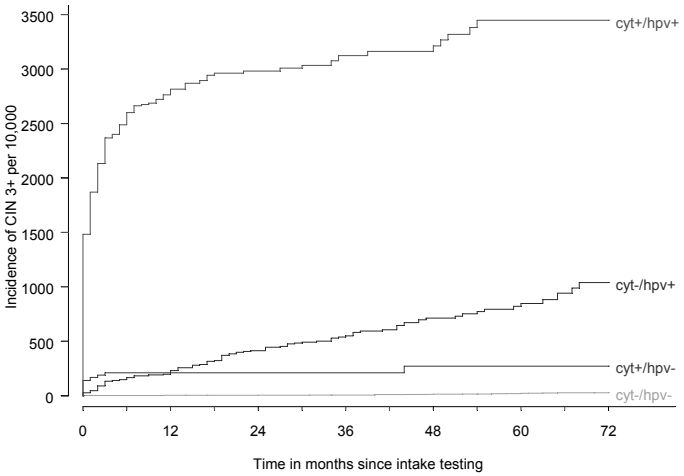

Figure 5-1. Kaplan-Meier plots of CIR for CIN 3+ for women who were cyt-/HPV-, cyt-/HPV+, cyt+/HPV-, and cyt+/HPV+ at baseline, in the first 72 months of follow-up. All 7 countries.

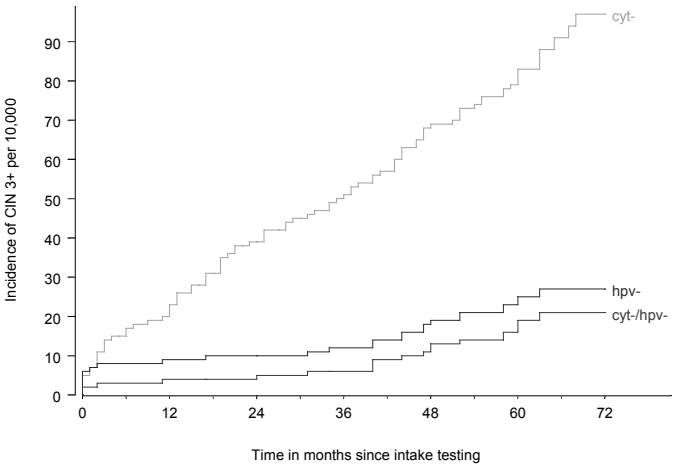

Figure 5-2. Kaplan-Meier plots of CIR for CIN 3+ for women who were cyt-, HPV-, and cyt-/HPV- at baseline, in the first 72 months of follow-up. Denmark and Tuebingen excluded.

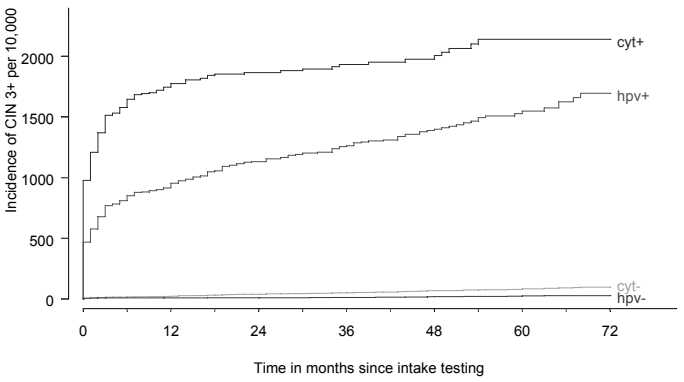

Figure 5-3. Kaplan-Meier plots of CIR for CIN 3+ for women who were cyt-, cyt+, HPV-, and HPV+ at baseline, in the first 72 months of follow-up. Denmark and Tuebingen excluded.

Table 5-3. Test characteristics (95% CI) at 72 months, by age.

		Overall	>49	35-49	<35	P†	30-34‡	<30‡	P‡
Cytology	**Cyt- at baseline**	20,078	3,380	10,308	6,390		982	2,080	
	- Of which CIN 3+	127	9	48	70		14	21	
	Cyt+ at baseline	1,273	161	557	555		111	330	
	- Of which CIN 3+	242	33	112	97		25	48	
	Sensitivity	0.599 (0.348-0.683)	0.760 (0.504-0.903)	0.641 (0.506-0.747)	0.431 (0.137-0.615)	0.034	0.642 (0.511-0.773)	0.524 (0.389-0.669)	0.268
	Specificity	0.954 (0.930-0.977)	0.964 (0.960-0.970)	0.959 (0.940-0.979)	0.937 (0.891-0.974)	0.546	0.924 (0.915-0.934)	0.875 (0.864-0.884)	<0.001
	NPV	0.990 (0.987-0.995)	0.997 (0.995-0.999)	0.993 (0.990-0.997)	0.979 (0.965-0.990)	0.008	0.981 (0.971-0.990)	0.975 (0.960-0.987)	0.479
	PPV	0.211 (0.102-0.291)	0.198 (0.048-0.915)	0.227 (0.113-0.342)	0.200 (0.087-0.269)	0.924	0.296 (0.191-0.403)	0.160 (0.123-0.197)	0.021
HPV	**HPV- at baseline**	18,552	3,169	9,740	5,643		876	1,683	
	- Of which CIN 3+	30	4	16	10		2	4	
	HPV+ at baseline	2,799	372	1,125	1,302		217	727	
	- Of which CIN 3+	339	38	144	157		37	65	
	Sensitivity	0.896 (0.796-0.949)	0.850 (0.452-0.986)	0.895 (0.749-0.971)	0.886 (0.778-0.966)	0.896	0.900 (0.785-0.965)	0.641 (0.433-0.873)	0.044
	Specificity	0.893 (0.929-0.942)	0.912 (0.872-0.957)	0.914 (0.861-0.944)	0.847 (0.747-0.926)	0.405	0.921 (0.914-0.929)	0.873 (0.861-0.882)	<0.001
	NPV	0.997 (0.996-0.999)	0.999 (0.995-1.000)	0.998 (0.996-1.000)	0.994 (0.988-0.999)	0.180	0.997 (0.993-0.999)	0.987 (0.974-0.997)	0.064
	PPV	0.171 (0.127-0.214)	0.106 (0.040-0.149)	0.166 (0.100-0.219)	0.196 (0.141-0.271)	0.106	0.254 (0.179-0.333)	0.129 (0.097-0.168)	0.008
Cytology +HPV (at least 1 positive)	**Cyt-/HPV- at baseline**	18,116	3,084	9,502	5,530		850	1,640	
	- Of which CIN 3+	20	3	8	9		2	4	
	Positive at baseline	3,235	457	1,363	1,415		243	770	
	- Of which CIN 3+	349	39	152	158		37	65	
	Sensitivity	0.921 (0.841-0.964)	0.897 (0.621-0.986)	0.935 (0.826-0.991)	0.993 (0.792-0.968)	0.695	0.954 (0.893-0.988)	0.845 (0.694-0.962)	0.113
	Specificity	0.872 (0.810-0.925)	0.887 (0.850-0.934)	0.892 (0.837-0.931)	0.829 (0.729-0.914)	0.452	0.813 (0.800-0.830)	0.694 (0.666-0.714)	<0.001
	NPV	0.998 (0.996-0.999)	0.999 (0.997-1.000)	0.999 (0.997-1.000)	0.994 (0.987-0.999)	0.126	0.997 (0.993-0.999)	0.988 (0.975-0.997)	0.097
	PPV	0.147 (0.099-0.190)	0.089 (0.038-0.127)	0.144 (0.081-0.204)	0.178 (0.125-0.248)	0.071	0.224 (0.151-0.294)	0.122 (0.092-0.157)	0.016

Denmark and Tuebingen excluded (data only known for one baseline group). †P-value for trend. ‡P-value for trend. As only the studies in France and Spain contained women below 30 years of age with complete test data, the data for 30-34 and <30 years of age as well as the trend test marked with ‡ is restricted to these cohorts only.

Analysis using an alternative outcome definition that included all high-grade lesions (CIN grade 2 or worse; CIN2+) found essentially similar results, but based on a higher number of cases (n=585). E.g., at 6 years of follow-up, the CIR of CIN2+ was 0.67% (95% CI: 0.39-1.11%) among HPV-negative women and 1.76% (95% CI: 1.00-2.47%) among cytology-negative women. The CIR of CIN2+ at 3 years of follow-up was 0.79% (95% CI: 0.43-1.16%) for cytology-negative women and 0.19% (95% CI: 0.07-0.38%) for HPV-negative women.

As the prevalence of HPV infection is highly age-dependent and since cytological performance also varies with age, we analysed PPV, NPV, sensitivity and specificity of the screening tests stratified by age group (Table 5-3). The sensitivity and NPV of cytology improved as women became older (Table 5-3). Both cytology and the HPV test had higher specificity for women above 35 years of age, but did not improve any further among women above 49 years of age (Table 5-3).

The seven studies included in the pooled analyses had estimates of CIR for CIN3+ that were not significantly different among cyt-/HPV-, cyt-/HPV+ or cyt+/HPV- women (scale parameters: 2.48, 1.80, 2.23; P-values: 0.14, 0.36, 0.1). However, the CIR of CIN3+ among women with positive cytology and HPV-test was clearly different between studies (scale parameter 4.77; P=0.01) (Figure 5-4).

5.5. Discussion

Using pooled data from seven HPV screening studies in six European countries we made a European estimate of the CIR for future histologically confirmed CIN3+ during 6 years of follow-up. The uniformly low CIR among cyt-/HPV- women suggests that double negativity confers a long-lasting protective effect that is remarkably robust, considering that the participating studies used several different types of HPV tests in several different settings and in several different age groups. The long-lasting protective effect was similarly low for HPV-negative women as for double negative women.

The fact that several studies in different settings in different countries and with different infrastructure and intensity of follow-up gave largely similar results is a strength of the study, as it implies that the data is generalizable to a variety of different settings. Similarly, the fact that the actual cytology tests and actual HPV tests that are being used in the different countries were studied implies that the data is generalizable. E.g., the largest study in the joint cohort (France) used the most modern cytology technique (liquid-based cytology) and the several other cohorts used the same routine conventional cytology as is being used in organised programs with documented cancer-preventive effect.

Our results are well in line with the results from a US cohort of 20,810 women that found that cyt-/HPV- women had a cumulative incidence of CIN3+ of 0.16 % after 45 months and 0.79 % after 122 months.[184] Similarly, a German cohort of 4,034 women reported that 0.7% of cyt-/HPV- developed CIN3+ during 5 years of follow-up[182] and a Dutch cohort of 2,810 women found only 1 case of CIN3+ among double negative women during 4.6 years of follow-up.[199]

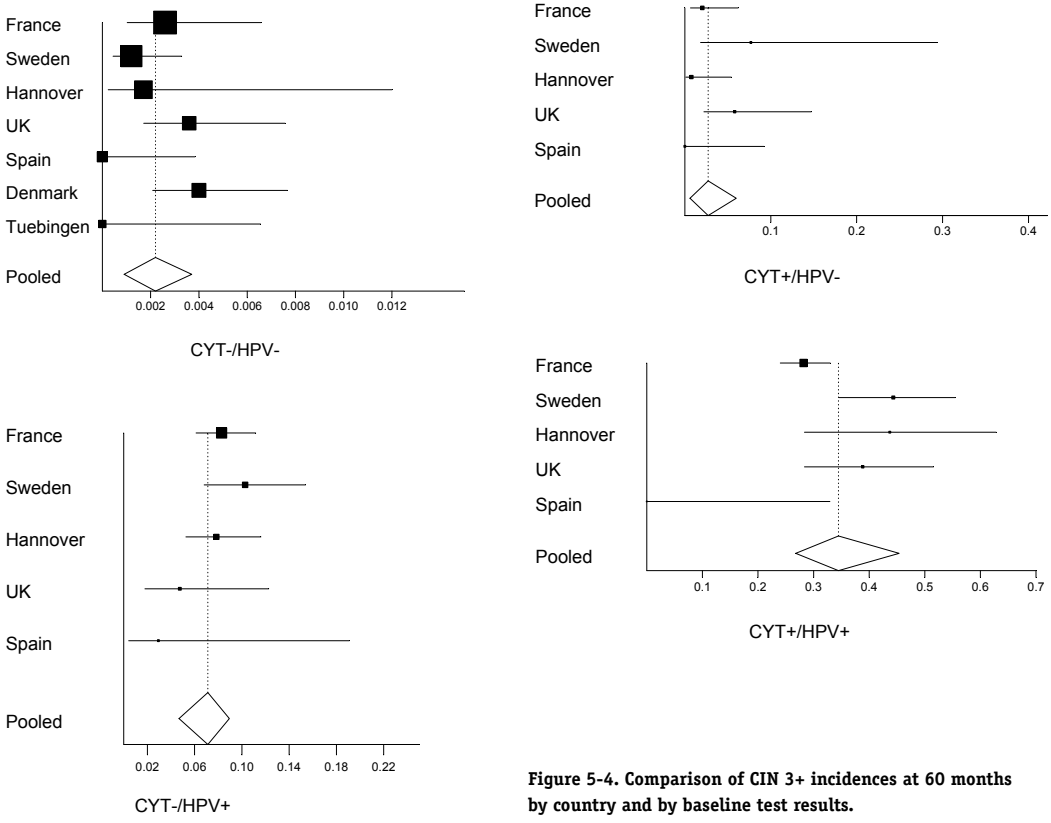

Figure 5-4. Comparison of CIN 3+ incidences at 60 months by country and by baseline test results.

As expected, the HPV test was less specific than cytology. The higher specificity above 35 years of age suggests that restricting HPV testing to older women would reduce overdiagnosis. However, with increasing length of follow-up the CIR for CIN3+ increased more among HPV-positive women than among cytology-positive women. This implies that the problem of HPV-based screening resulting in increased overdiagnosis with women unnecessarily referred to clinical procedures is attenuated in evaluations with longer follow-up – some of the HPV positivity that appears to be false positivity in cross-sectional evaluations will turn out to be true, but earlier, detection of CIN 3+ cases.

Verification bias may overestimate the performance of screening tests when only women with a positive screening test are referred to colposcopy. Only some of the included studies performed colposcopies of double-negative women. However, it is rare to diagnose CIN3+ by colposcopy of double-negative women[187, 200] and the fact that there was limited variability between studies also suggests that verification bias has not materially affected our estimates.

Nevertheless, as assessment of the CIN3+ incidence by baseline group during follow-up is dependent on the screening intensity our estimates should be interpreted as relative rather than absolute.[201] However, we only included women who had been screened at least once during follow-up and the follow-up time was longer than the recommended screening intervals in all the included studies.[111]

In one study (Spain), there was no action taken because of positive baseline HPV tests whereas four studies mandated extra testing and/or colposcopy after baseline HPV-positivity (Sweden, Hannover, UK and France). As Denmark and Tuebingen are not included in the follow-up of cytology-positive or HPV-positive women, the CIR of CIN3+ presented in this study are almost entirely based on active follow-up of HPV-tests and should reflect the outcome of active HPV-based screening strategies.

As CIN3+ prevalence is associated with HPV prevalence it is possible that some heterogeneity between studies is explained by differences in HPV prevalence e.g. Spain has been reported to have a low prevalence of HPV.[194] Another possible source of variability is the fact that the German, French, Danish, British and Spanish studies used HC2 for HPV detection while the Swedish study used PCR. The agreement of HC2 and PCR has been reported to be substantial[202, 203] and there was also no obvious difference in results depending on the HPV test used. The most obvious source of heterogeneity between countries was the variability in Pap smear interpretation, as the proportions of cytology positives ranged from 2% (Sweden) over 4% in Hannover and Spain to 5% in the UK and 7% in France; these differences cannot be entirely explained by the observed differences in the HPV prevalence.

In conclusion, we have provided joint European data suggesting that screening intervals could safely be lengthened to 6 years among women with a negative HPV test. This could at least partly compensate for the increased referral rate resulting from HPV-based screening strategies.

Acknowledgments

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CHAPTER 6. Triage Using HPV-testing in Persistent Borderline and Mildly Dyskaryotic Smears: Proposal for New Guidelines

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6.1. Abstract

In the Netherlands 2% of cervical smears in the cervical cancer screening program are read as borderline or mildly dyskaryotic cytology (BMD smear). Only in about 10% of these women a high-grade CIN lesion (CIN 2-3) is present, therefore referral is for the majority unnecessary. In this study triage with high-risk HPV (hrHPV) testing was used to identify women at risk for development of high-grade CIN lesions after a repeat BMD smear. A "wait-and-see" period was incorporated allowing clearance of HPV and regression of the lesion. Women with a low-grade lesion, irrespective of their HPV-status were monitored at 12 months; women with a high-grade lesion at 6 and 12 months. Fifty-one of the 105 women (49%) were hrHPV negative at baseline, none of them showed progression of the lesion within the first year of follow-up (NPV 100%). High-grade CIN was present in one patient who was HPV negative at baseline (2%), she demonstrated regression after 12 months. Nineteen of the hrHPV positive women (35%) demonstrated a high-grade CIN lesion at baseline, three cleared hrHPV after 6 months, with a subsequent regression of CIN. Ten women remained hrHPV positive with persistence of high-grade CIN and were eventually treated. At baseline 35 hrHPV positive women demonstrated a low-grade lesion, 19 remained hrHPV positive after 12 months, 5 developed high-grade CIN. Sixteen out of the 35 cleared the hrHPV infection without progression of the lesion. In conclusion, triage using hrHPV testing for women with persistent BMD cytology can select women who are not at risk for development of high-grade CIN. We recommend return to the screening program without referral for colposcopic examination if hrHPV is absent. For hrHPV positive women a repeat hrHPV test after another six months is suggested. Referral is only required if persistence of hrHPV is established.

6.2. Introduction

In the Dutch population-based cervical cancer-screening program women between 30 and 60 years of age undergo a cytological smear every fifth year. Recent surveys showed that about 2% of these smears contained borderline or mildly dyskaryotic (BMD) changes.[15, 60] According to current screening guidelines, in these cases cytology is repeated after 6 months and (if negative) after 12 months. When cytology is persistently abnormal, women are referred to the gynaecologist where colposcopic examination is performed and a biopsy is taken for histological examination. When histology shows no or low-grade dysplasia (≤CIN 1) women will be kept under cytological surveillance without immediate treatment. High-grade dysplasia (CIN 2-3) or a worse lesion will be treated. Only ten percent of women with a single BMD smear show a high-grade CIN lesion.[37, 41, 204, 205] Consequently, there is a need for an improved risk assessment of women with BMD.

Infection with high-risk HPV (hrHPV) has been established as an important etiological factor in the carcinogenesis of cervical cancer.[5, 8] Persistence of hrHPV infection is required for development of cervical cancer.[25, 27, 191, 206] Several studies have shown that HPV testing has a high sensitivity (approximately 95%) for identifying high-grade lesions and cervical carcinoma. Moreover, the negative predictive value of HPV testing for detection of a high-grade CIN lesion or even (micro) invasive carcinoma is nearly 100%.[101, 207] Consequently, HPV-testing may play an important role in clinical practice, i.e. in triage of women with BMD cytology, as an adjunct to cytology in primary screening and in post-treatment protocols.[208-210]

The prevalence of hrHPV in women with BMD smears is about 35%[211, 212] and it is likely that hrHPV negative BMD women are not at risk for development of high-grade CIN lesions or carcinoma. Hence, triage with hrHPV testing may improve the selection

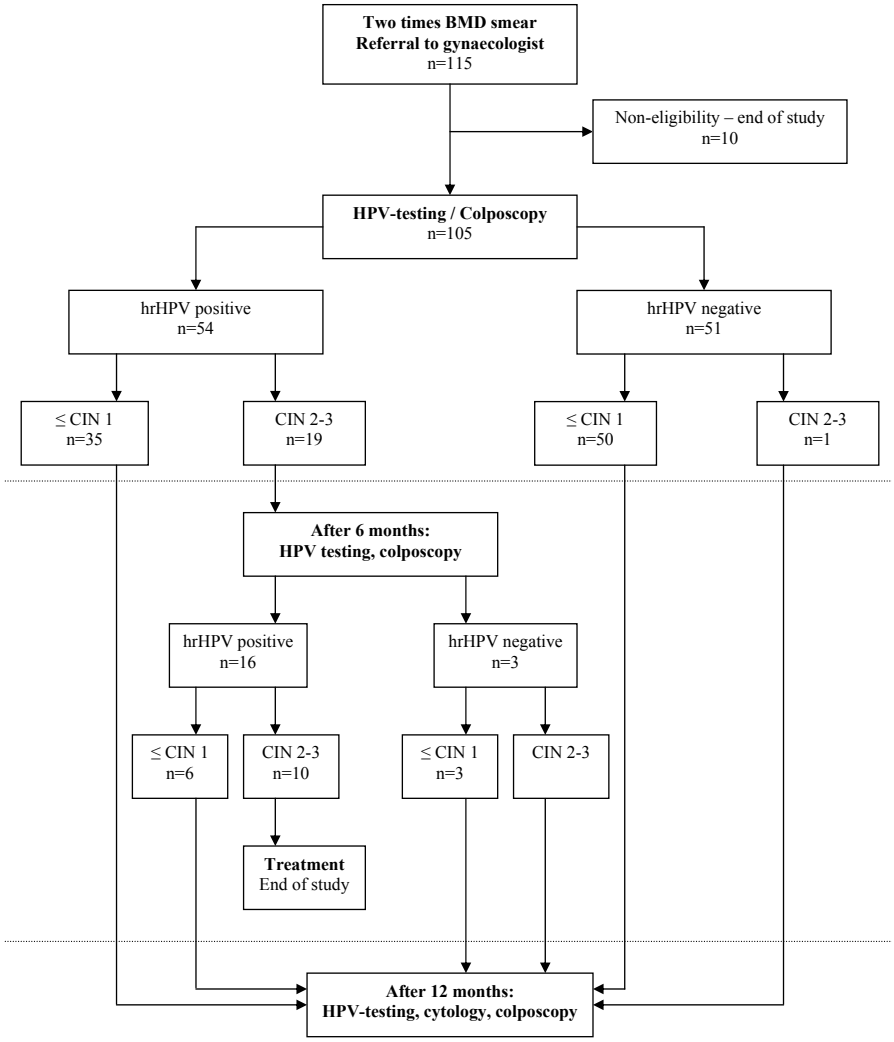

Figure 6-1. Trial design.

of women at risk for development of cervical cancer and consequently the management of women with BMD smears.[44, 106, 209, 211, 213, 214] This would not only have the advantage of improving the efficiency of the screening program through fewer referrals, but also reduce unnecessary anxiety among the majority of women with BMD who are not at risk for high-grade CIN.[204, 205, 215]

In this prospective study, triage with hrHPV testing was performed in the follow-up of women referred with a repeated BMD established in the screening program. Unlike most published studies a wait-and-see period was incorporated in the present protocol to allow potential clearance of hrHPV infection and consequently regression of the cervical lesion. We evaluated the use of triage with hrHPV testing to prevent unnecessary diagnostic procedures and treatment.

6.3. Material and Methods

All women with a repeated BMD smear referred through the national screening program to gynaecological outpatient clinics were asked to participate in this study. Participating hospitals were the Erasmus University Medical Centre Rotterdam (December 1999 - May 2003), the Hospital Walcheren in Vlissingen (January 2000 - March 2002) and the VU University Medical Center Amsterdam (June 2000 - January 2003). Exclusion criteria were pregnancy at time of enrolment or during follow-up (n=3), non-Hodgkin lymphoma (n=1), age younger than 30 years or older than 60 years at time of abnormal smear (n=5), or insufficient Dutch or English language skills (n=1).

Figure 6-1 shows the trial design. At baseline women were asked to complete a questionnaire on education, ethnic background, smoking, number of sexual partners, sexarche, contraceptive use and history of sexually transmitted diseases. A cervical scrape was taken for HPV detection followed by colposcopy. Standard colposcopical assessment with acetic acid and iodine solution was performed by expert gynaecologists. Biopsies were taken from all colposcopic abnormalities. At this first visit no treatment was carried out.

Table 6-1. Baseline characteristics.

	Total	hrHPV negative	hrHPV positive	Significance
	n=105 (100%)	n=51 (100%)	n=54 (100%)	
Age at intake (years)†				P<0.01
30-40	58 (55%)	17 (33%)	41 (76%)	
40-50	26 (25%)	16 (31%)	10 (19%)	
50-60	21 (20%)	18 (35%)	3 (6%)	
Histology at intake†				P<0.01
Low-grade	85 (81%)	50 (98%)	35 (65%)	
High-grade	20 (19%)	1 (2%)	19 (35%)	
Ethnic background				
Caucasian	82 (78%)	43 (84%)	39 (72%)	
Asian	4 (4%)	2 (4%)	2 (4%)	
Negroid	10 (10%)	4 (8%)	6 (11%)	
Mediterranean	4 (4%)	0	4 (7%)	
Other	5 (5%)	2 (4%)	3 (6%)	
Education				
Primary or less	9 (9%)	4 (8%)	5 (9%)	
Secondary, incomplete	55 (52%)	32 (63%)	23 (43%)	
Secondary or more	41 (39%)	15 (29%)	26 (48%)	
Age at first intercourse (years)				
≤15	23 (22%)	8 (16%)	15 (28%)	
16-18	51 (49%)	26 (51%)	25 (46%)	
≥19	31 (30%)	17 (33%)	14 (26%)	
No. of sexual partners last year				
0-1	89 (85%)	45 (88%)	44 (81%)	
2-4	16 (15%)	6 (12%)	10 (19%)	
Smoking				
No	56 (53%)	32 (63%)	24 (44%)	
Yes	49 (47%)	19 (37%)	30 (56%)	
Oral contraceptive use				
No	62 (59%)	34 (67%)	28 (52%)	
Yes	43 (41%)	17 (33%)	26 (48%)	
History of Sexually Transmitted disease†				P<0.01
No	84 (80%)	48 (94%)	36 (67%)	
Chlamydia trachomatis	9 (9%)	3 (6%)	6 (11%)	
Condylomata accuminata	5 (5%)		5 (9%)	
Other	7 (7%)		7 (13%)	

†Fisher's Exact P< 0.01.

Women who were hrHPV negative were reviewed after 12 months. HrHPV positive women were reviewed after 12 months if the colposcopically-directed biopsy was ≤CIN 1, and after 6 and 12 months if histology showed moderate to severe dysplasia (CIN 2-3). During follow-up visits hrHPV-testing and colposcopy were performed. Women with a persistent hrHPV-positive high-grade CIN lesion during follow-up were treated by loop excision of the transformation zone (LETZ). The study endpoint was reached after 12 months, or after treatment, whichever came first. The study protocol was approved by a multicenter research ethics committee and by local committees at all three hospitals. All women voluntarily gave signed informed consent before enrolment.

Histology

Lesions were histologically defined as mild dysplasia (CIN 1), moderate dysplasia (CIN 2), severe dysplasia (CIN 3) and (micro) invasive cancer. Lesions ≤CIN 1 are hereafter referred to as a low-grade lesion and CIN 2-3 as a high-grade lesion. Regression or progression was defined as histological change from a high-grade lesion to low-grade lesion or vice versa, detected in the biopsy material at two consecutive time points as scheduled by the trial design. All histological samples were read by expert pathologists.

Human papillomavirus testing

All HPV samples were taken by a cervical biosampler (Accellon Combi® Medscand Medical, Sweden). Testing for HPV was conducted by using a general/consensus primer based GP5+/ GP6+ polymerase chain reaction (PCR) enzyme immunoassay (EIA) for 14 high risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).[216] This test has been clinically validated.[206, 217, 218] Additionally, reverse line blot (RLB) analysis was used

to identify individual HPV types in case the PCR-EIA was positive.[216] We used β-globin PCR to identify sampling errors and to monitor for PCR inhibitors.

Statistical analysis

The risk of development of high-grade CIN associated with hrHPV presence was assessed with the Fisher exact test and Chi-square. Confidence intervals of 95 percent and 2-sided P-values were used.

6.4. Results

A total of 105 women met the inclusion criteria. The mean age was 39 years (range 30-60 years). The median time-lag between the two BMD smears on the basis of which women were referred was 7 months, with a range of 2-20 months. The median time-lag between the second BMD smear and colposcopic examination was 2 months (range 0-8 months).

At baseline 54 women (51%) were hrHPV positive, leaving 51 hrHPV negative subjects (Figure 6-1). HPV 16 and 31 were the most frequently detected hrHPV types (40% and 20% of all infections, respectively). Other types were less common: HPV 18 (7%), HPV 33 (10%), HPV 35 (7%), HPV 42 (7%), HPV 45 (2%), HPV 51 (7%), HPV 52 (10%), HPV 56 (10%), HPV 58 (7%), HPV 59 (2%) and HPV 66 (2%). Of the observed HPV positive scrapings at baseline, 68% contained single infections and in the remaining 32% a multiple infection was detected. With one exception, all hrHPV negative women demonstrated no or a low-grade lesion at baseline biopsy: 27 women without dysplasia (53%) and 23 women with CIN 1 (45%). One hrHPV negative woman had a CIN 2 lesion at baseline.

Among hrHPV positive women at baseline, 35 cases (65%) had ≤CIN 1 and 19 cases (35%) CIN 2-3. The first group consisted of 10 women (19%) without dysplasia and 25 women (46%) with a CIN 1 lesion, whereas the high-grade CIN group comprised 14 women (26%) with a CIN 2 lesion and five women (9%) with a CIN 3 lesion.

Table 6-1 shows the characteristics of the study population stratified according to hrHPV presence at baseline. When age is stratified in three categories 30-40, 40-50 and 50-60 years respectively, we see a significant difference for the youngest group (30-40 years) where hrHPV is more frequently present (P<0.01). The presence of CIN 2-3 was significantly higher in women who were hrHPV positive at baseline, compared to hrHPV negative women (P<0.01 with an odds ratio of 27 (95% CI: 3-211)). Women who were hrHPV negative were less likely to have a history of sexually transmitted disease (P<0.01). Other risk factors such as education, ethnic background, sexarche, number of sexual partners in the preceding year, smoking and oral contraceptive showed no difference of statistical significance between groups with and without HPV infection.

Follow-up of hrHPV negative women

One woman acquired hrHPV infection after 12 months, without progression to high-grade CIN. All other women (50/51) remained hrHPV negative and did not develop a high-grade CIN lesion. The woman with a high-grade CIN lesion at baseline showed histological regression to no dysplasia after 12 months (Table 6-2).

Follow-up of hrHPV positive women

Nineteen of the 35 hrHPV positive women (54%) with ≤CIN 1 at baseline demonstrated persistence of hrHPV infection after 12 months. Five out of these 19 (26%) showed progression to high-grade CIN. No (micro) invasion was detected. Fourteen women without hrHPV clearance continued to have ≤CIN 1. Clearance of hrHPV infection occurred in 16/35 women (46%), none of whom showed progression detected in histological biopsies, i.e. 14 women without dysplasia and 2 women who still had CIN 1 after 12 months.

Among the 19 women with a high-grade lesion three (16%) cleared the hrHPV infection after six months, with histological regression to a low-grade lesion, and remained hrHPV negative after 12 months. Amongst the 16 women who were still hrHPV positive after 6 months (84%) 10 (63%) revealed a (persistent) high-grade lesion. No (micro) invasion was detected. These 10 women were treated. The remaining six women (37%) demonstrated regression to a low-grade lesion. One of them was treated at her own request and therefore left the study group prematurely. After 12 months two out of five women remained persistently hrHPV positive, one of whom had a high-grade lesion (CIN 2). The other three women showed clearance of hrHPV and a low-grade lesion on histology.

6.5. Discussion

In this study progression to a high-grade CIN lesion was not seen in hrHPV negative women with a persistent BMD smear detected in the national screening program. All women with a high-grade lesion were hrHPV positive at baseline, except for one woman who had a CIN 2 lesion at baseline and was hrHPV negative. Histology from this patient showed regression to a low-grade lesion after 12 months, suggesting that for this woman clearance of hrHPV was already evident at baseline, with subsequent regression of the lesion becoming apparent after 12 months. This corresponds with the findings of other studies.[26, 106, 208]

In our study, the negative predictive value (NPV) of hrHPV testing for having high-grade CIN was 98% at baseline for women with a repeated BMD smear, and 100% after 6 and 12 months follow-up. This suggests that hrHPV negative women, despite a persistent BMD cytology, are not at risk for high-grade CIN.

Various studies have shown that better results are obtained using HPV detection when compared with conventional cytology

Table 6-2. Histology outcome related to HPV status at baseline.

	Baseline			t=6 months†			t=12 months		
	≤CIN 1	CIN 2-3	Total n (%)	≤CIN 1	CIN 2-3	Total n (%)	≤CIN 1	CIN 2-3	Total n (%)
HPV-positive (n)	35	19	54 (51%)	9	10	19 (100%)	37	6	43 (41%)
HPV-negative (n)	50	1	51 (49%)	-	-	-	51	0	51 (49%)
Total (n)	85	20	105	9	10	19	88	6	94‡

†Only HPV positive CIN 2-3 had t=6 months follow-up. ‡11 women treated (10%).

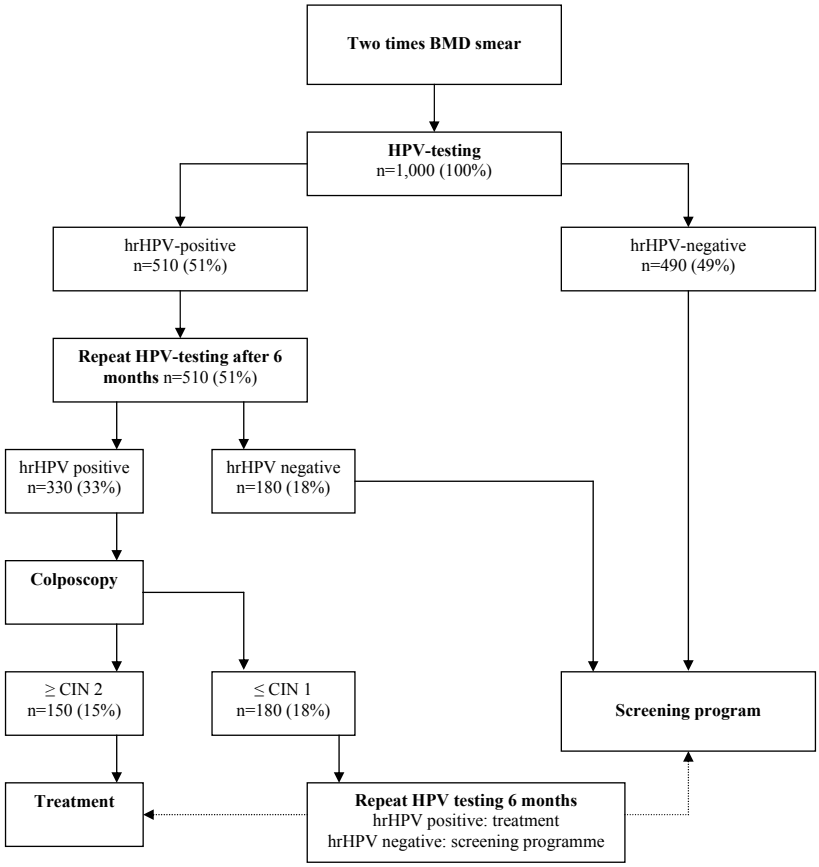

Figure 6-2. Policy proposal, illustrated on a hypothetical number of 1,000 patients. Reduction of 67% referral for colposcopic evaluation (49 + 18), 9 percentage points or 9/24*100=38% less treatment (current directions 25/105 (24%) treated vs. proposed proposal 16/105 (15%) treated) accomplished if 151% HPV-testing is added.

(i.e. NPV cytology 93-98%).[5, 101, 211] Triage with HPV-testing as an adjunct to cytology has been proposed for women with minimal cytological changes.[208, 209, 213] Several studies have used different classifications of mildly abnormal cytology.[106, 208, 211, 213, 219-221] Cuzick et al. included women with borderline cytology and negative cytology but hrHPV positive (HART study).[213] The ALTS study describes triage in women with ASCUS and LSIL cytology (ALTS Group).[222] An overview of triage studies with corresponding data is shown in Table 6-3. Selection was based on HPV detection methods that were clinically validated in longitudinal studies (i.e. HCII and GP5+/6+ PCR), since the clinical rather than the analytical performance of HPV detection methods should be considered for triage policies.[223] The main benefit that can be concluded from all listed studies is a reduction in referral of hrHPV negative women with minimal abnormal cytology for colposcopy. For more severe cytological abnormalities the use of triage with HPV-testing is not indicated. Our study was based on women referred to the gynaecologist for two times BMD smear according to the national guidelines. Based on our results we suggest that women with a persistent BMD smear and a concomitant negative hrHPV test result are not at risk for development of cervical cancer and should stay in the screening program, without colposcopic evaluation. In the Netherlands the next scheduled screening would then take place after 4.5 years. The risk that a woman will develop high-grade CIN within that period of time seems acceptable, since it will be exceptional to take place within 5 years given that a persistent hrHPV infection is required.[37, 100] Precursor lesions are detectable within an average of 10-15 years before progression to cancer.[34] Thus, even though our study only covers 12 months of follow-up, it seems reasonable to return women with a hrHPV negative persistent BMD smear into the screening program without additional surveillance or treatment. However, an estimate of excess risk among hrHPV negative women with BMD cytology is expected within the next few years when e.g. the ongoing Dutch POBASCAM screening study and Swedish screening study are expected to yield final results.[15, 138]

In our study 54/105 women (51%) were hrHPV positive at baseline of whom 19 (35%) had a histologically confirmed high-grade CIN lesion. The overall frequency of high-grade CIN was 19%, which is comparable with the findings of Ho et al.[106] In contrast, a previous study by our group demonstrated a prevalence of 10%.[109] In that study however, about half of the women were referred after one BMD smear in contrast to our current

Table 6-3. Triage in women with borderline or low grade cytological abnormalities (selected studies based on validated HPV detection methods).

Study	Population	n	Mean age (years)	Follow-up	HPV-positive	HPV clearance	CIN 2/3	Sens %	Spec %	PPV %	NPV %	Reduction of referral
(HART)				RCT								
Cuzick et al. 2003	Borderline cytology negative cytology HPV-positive	825	42	6-12 months	27%	35-45%	3%	97	93	13		HPV-negative borderline cytology return to screening HPV-positive borderline cytology retest HPV after 12 months HPV-positive mild dyskaryosis refer
Ho et al. 2003	repeated mild dyskaryosis or less	149	28-32	Observational study	59%	44%	24%	100				42% reduction based on HPV-positive
				Every 6 months								HPV-negative mild dyskaryosis retest 6-12 months
				Mean 3 years								retest HPV: total of 67% less referral
(ALTS)				RCT								
Solomon et al. 2001	ASCUS	2198	29	6-12-18 months	54%		12%	96		20	99	44% reduction based on ASCUS HPV-positive
Sherman et al. 2002	ASCUS-LSIL	3046	29-25		89%			96-97				46% reduction based on ASCUS HPV-positive, 15% reduction if LSIL HPV-positive
Guido et al. 2003	ASCUS	881	25	≤CIN 1 follow-up			18%	92				45% reduction based on HPV retest after 12 months
Cox et al. 2003	ASCUS HPV-positive	1132			51%		18%					49% reduction based on ASCUS HPV-positive
	LSIL	852			81%		18%					
Zielinski et al. 2001	single and repeated borderline or mild dyskaryosis	278	41	Observational study	42%	45%	10%	96	63	22	98	58% reduction based on HPV-positive
				Every 6 months Median 1.4 years								
Current study	repeated borderline or mild dyskaryosis	105	39	Intervention study: treatment postponement 6-12 months	51%	41%	19%	95	59	35	98	49% reduction based on HPV-positive

study where women were referred after two BMD smears in 6 months. Consequently, this group with BMD contains more women with mild dyskaryosis than the previous group (38% vs. 26%) (data not shown). Therefore, our results can be explained by the fact that the prevalence of high-grade CIN increases with severity of abnormal cytology.[27, 211]

In this trial women with a high-grade lesion were not treated immediately. A "wait-and-see" period of six months showed persistence of high-grade CIN only in women with persistent hrHPV infection. No patient developed (micro) invasive carcinoma. These findings suggest that a wait-and-see period for (at least) six months involves no additional health risks. In addition, 16% of these women showed clearance of hrHPV with a subsequent regression of the lesion. They were no longer at risk of development of high-grade CIN, and consequently, an expectative policy could result in a reduction of the need for treatment.

The majority of hrHPV positive women with a repeat BMD smear have low-grade CIN lesions (in our study 35 out of 54 (65%) at baseline). This is in agreement with the findings of previous studies.[41, 106, 208, 211, 213, 219-221] Approximately half of these women cleared hrHPV in 12 months and none of them developed a high-grade CIN during this time. Fourteen percent developed high-grade CIN after 12 months (all were persistently hrHPV positive), without an observed development of (micro) invasive carcinoma. It should, however, be considered that the biopsies taken to establish the severity of premalignant lesions may have interfered with the natural course of disease. On the other hand, several natural history studies have demonstrated that HPV clearance and histological regression occur after 6-12 months.[26, 204, 224] Our follow-up time of 12 months should therefore cover the major part of regression. Consequently, referral can therefore be restricted to women with a repeat BMD smear who remain persistently hrHPV positive for at least another six months.

Although our proposal requires more hrHPV testing (100%), according to the present study it will lead to a 49% reduction in referrals for colposcopy. A 'wait-and-see' period in the HPV positive group for at least six months will result in a supplement of 51% HPV-testing and a further 18% reduction in referrals and 9 percentage points treatment reduction. In summary, a total reduction of 67% referrals for colposcopic evaluation and 38% less treatment can be obtained by addition of 151% HPV-testing. The 6 months wait-and-see period in hrHPV positive women may also have negative influences on the women involved. Some women might prefer a see-and-treat policy instead of a more conservative approach. Proper information and explanation of the natural development of premalignant lesions in relation to hrHPV infection by the physician is therefore required, after which patients preference may influence further choices especially in this group.[30] The potential gain of less treatment however, remains to be weighted against potential changes in quality-of-life and effectiveness. Our recommendations are summarised in Figure 6-2.

In conclusion, triage using hrHPV testing for women with a persistent borderline or mildly dyskaryotic cytology is recommended. Women can stay in the screening program without referral to the gynaecologist if no hrHPV infection can be determined. We suggest a repeat HPV test after another six months for hrHPV positive women. Referral for colposcopy is only required if persistence of hrHPV is demonstrated. Women who cleared hrHPV infection are no longer at risk of development of premalignant lesion. Further surveillance can take place within the screening program at the protocollized interval of five years.

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CHAPTER 7. Human Papillomavirus Triage of Women with Persistent Borderline or Mildly Dyskaryotic Smears: Comparison of Costs and Side Effects of Three Alternative Strategies

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7.1. Abstract

The conventional direct referral to colposcopy of persistent borderline or mildly dyskaryotic (BMD) smears in cervical cancer screening leads to considerable unnecessary referrals and associated anxiety and costs. This may be improved by including testing for oncogenic human papillomavirus (HPV) in the triage. We assessed costs and side effects (referrals, treatments, time in follow-up) for three possible HPV triage strategies (immediate HPV testing, a 6-month delay in HPV testing, a two-stage combination of both) and compared them with the conventional strategy. The assessments are based on recent Dutch data from various national databases and trials. We estimated that the referral rate could be reduced by 49%, 58% and 58% with immediate, delayed and two-stage HPV testing, respectively. As a consequence, the average length of follow-up, as well as average costs, also decrease. Therefore we advocate including HPV testing before referring to colposcopy. Among the three HPV strategies, analysis of additional aspects favours implementation of immediate HPV testing.

7.2. Background

Cytology-based cervical cancer screening prevents deaths by treating pre-invasive (cervical intraepithelial neoplasia - CIN) and early invasive disease. Low-grade abnormalities described as borderline and mild dyskaryosis (BMD) are the most common type of cytologic abnormalities, ranging in frequency from below 2% in the Netherlands to above 6% in Finland.[Chapter 2] In several countries, it is currently recommended to follow up these women with a Pap smear in 6 months.[49, 102, 103] Women are then referred for a colposcopy if the BMD abnormality does not normalise.[49] In the Netherlands, about one third of the women with BMD primary screening smears is eventually referred,[Chapter 2] most often because of BMD persistence and less often because of cytological progression.[225]

This diagnostic policy of following-up BMD smears induces a considerable amount of side effects in terms of a high number of referrals and a long follow-up period with associated costs and psychologic consequences. Sixty to ninety percent of women with BMD persistence have no high-grade lesion that needs to be treated.[104-109, 226, Chapter 6] The burden on women and the health care could be reduced if the subgroup at high risk for a significant lesion were identified.

It has been consistently shown in the literature that detection of an infection with one of the high-risk human papillomavirus types (HPV) can be used for a risk stratification of women with low-grade abnormal smears.[101, 208] An infection with HPV is a necessary factor in the development of invasive cervical cancer.[5] Because no histological progression is seen in women who spontaneously clear the HPV infection,[5] women without a detectable HPV infection do not need further follow-up. That could be the case in 40-60% of women with persistent BMD smears.[104-109, Chapter 6] The discussion about the optimal strategy and time points of incorporating HPV testing into triage of women with BMD smears is still on-going.[227-229]

To inform this discussion, we investigated the costs and the side effects of three strategies of managing women with persistent (defined as two consecutive) BMD smears using HPV testing as a triage tool. Both the side effects and the costs are compared to those associated with the conventional management (i.e., direct colposcopy of all women without first assessing their HPV status).

7.3. Methods

Triage strategies

We analysed three possible HPV triage strategies of women with two consecutive BMD smears (Figure 7-1; corresponding to ASC-US+ASC-H+LSIL in the Bethesda 2001 classification[40]). The difference among these strategies is in the timing of HPV testing, and the consequent referral to colposcopy:

- A – immediate HPV triage: the co-collected HPV sample is analysed immediately when BMD persistence is established (t=0), and all HPV-positive women are referred to colposcopy;
- B – delayed HPV triage: an HPV sample is collected six months after the second BMD smear (t=6), and all HPV positive women (i.e., those who have not cleared the virus) are referred to colposcopy;

– C – two-stage HPV triage: the co-collected HPV sample is analysed immediately when BMD persistence is established (t=0), HPV-positive women are re-tested for HPV at t=6, and all women who remain HPV-positive (i.e., those who have not cleared the virus) are referred to colposcopy.

We assumed that in strategies A and C the HPV samples are co-collected when the woman presents at the general practitioner (GP) to have a follow-up Pap smear after a BMD primary screening smear (both conventional cytology). These HPV samples are investigated if the follow-up smear is read as BMD. Women with one negative HPV test return to a normal screening schedule. The three HPV strategies were compared for side effects and costs to:

– D – conventional strategy: direct colposcopy, i.e. a referral at t=0 of all women with two consecutive BMD smears without a prior assessment of their HPV status.

Quantification of side effects and costs

We assessed the side effects and costs per woman with two consecutive BMD smears for the period after the second BMD smear. This period includes all HPV sampling for triage and the complete post-referral management.

In the Dutch cervical cancer screening programme, roughly 1 in 4 women with a primary BMD smear has a follow-up (=second) BMD smear; the remaining 3 in 4 women have either a negative follow-up smear, or a highly abnormal (>BMD) smear.[225] Because of co-collection at t=0 in strategies A and C, when the outcomes of cytologic testing are not yet known, HPV samples would need to be taken from all women with a primary BMD smear. This represents extra costs compared to the conventional strategy, and needs to be taken into account in the analysis. Therefore, in these two strategies we assumed that three extra HPV samples need to be collected for each woman with two consecutive BMD smears. We further assumed that only those collected samples that are relevant for our analysis, i.e. 1 in 4, are read in the laboratory.

The proportion of women referred, the proportion treated and the time needed to complete the recommended follow-up are quantified from epidemiological data from a recent Dutch trial performed at the Erasmus Medical Center (Erasmus MC) reported earlier.[Chapter 6] This trial aimed to evaluate the potential to prevent unnecessary diagnostic procedures and treatments by doing the HPV triage of women with two consecutive BMD smears corresponding to strategy C. At enrolment, the gynaecologist took an HPV sample and biopsies from all colposcopic abnormalities but treatment was deferred. Women with an HPV-positive high-grade (CIN 2/3) lesion at enrolment were followed-up 6 months later with an HPV test and a colposcopically guided biopsy. If the HPV-positive CIN 2/3 lesion persisted, the woman was treated. If instead within 6 months the woman cleared the virus or no CIN 2/3 lesion could be established anymore, she was seen at the exit visit 12 months after enrolment together with those women who tested HPV-negative at enrolment or had (at most) a low-grade lesion (CIN 0/1). At this exit visit, women were tested for HPV and underwent colposcopy. At enrolment, 51% of women had detectable HPV (Table 7-1). Nineteen percent of all enrolled women were found to have a CIN 2/3 lesion at t=0, i.e. 35% of all HPV-positive and 2% of HPV-negative women. The observed 12-month progression and persistence proportions of (untreated) CIN 0/1 lesions (CIN 0 to >CIN 0, and CIN 1 to ≥CIN 1; both transitions may prompt treatment during post-colposcopic follow-up) were dependent on the HPV status at enrolment (Table 7-2). No cancer was found during follow-up.

In the Erasmus MC trial, the HPV status and the distribution of CIN lesions at t=6 were not directly observed for all women. We applied a multi-state Markov model on the longitudinal data of all HPV-positive women at enrolment (n=54), using the HPV and CIN prevalence observed at t=0 (for all 54 women), at t=6 (for the subgroup with HPV-positive CIN 2/3 at enrolment), and at t=12 (for women with HPV-positive CIN 0/1 at enrolment, and women without persistent HPV-positive CIN 2/3 at t=6). The result of this model was an estimate of HPV and CIN prevalence for all 54 women at t=6. We used the *msm* package for the statistical program R version 0.6.3 (Christopher Jackson, Department of Epidemiology and Public Health, Imperial College, London). We allowed for the following transitions: HPV clearance, CIN progression and CIN regression, and assumed that women treated at t=6 would without treatment have remained in the same state until t=12. This lead to an estimate that 18% of HPV-positive women at t=0 cleared this infection by t=6 (Table 7-1). With the same model we also estimated that 44% of women who do not clear the HPV by t=6 have CIN 2/3.

For the HPV prevalence and persistence rates and the CIN 2/3 prevalence, we extracted the plausible ranges

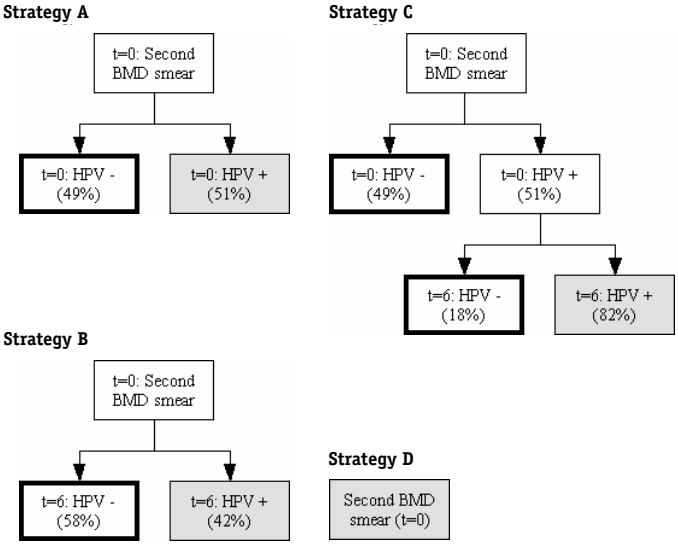

Figure 7-1. Strategies A, B, C, and D (conventional strategy) for triage of persistent BMD smears. Shaded rectangles represent women who need to be referred, whereas the rectangles with a bold border represent women who can return to the regular screening programme.

Table 7-1. HPV prevalence and CIN lesions.

	Base case	Range (Literature)	Outcomes affected
(1) HPV prevalence at t=0	51%[Chapter 6]	40-60%[104-109]	Equals the referral rate in strategy A.
(2) Persistence of HPV infection from t=0 to t=6	82%†	60-85%[106, 109]	Multiplied with *(1)*, it equals the referral rate in strategies B and C.
(3) CIN 2+ among women regardless of HPV status at t=0	19%[Chapter 6]	10-40%[104-109, 226]	Equals the proportion of women treated immediately upon referral in strategy D.
(4) CIN 2+ among HPV-positive women at t=0	35%[Chapter 6]	20-50%[106-109]	Multiplied with *(1)*, it equals the proportion of women treated immediately upon referral under strategy A.
(5) CIN 2+ among women with a persistent HPV infection at t=6	44%†	30-60%	Multiplied with *(2)*, it equals the proportion of women treated immediately upon referral under strategies B and C.
(6) CIN 0 : CIN 1 ratio, regardless of HPV status	44%:56%[Chapter 6]		Multiplied with (1 - *(3)*) and the respective progression rates (Table 7-2), it equals the proportion of women treated at follow-up in strategy D. Multiplied with the cost per CIN 0 or CIN 1 case (Table 7-4), determines the total cost of referral of CIN 0/1 in strategy D.
(7) CIN 0 : CIN 1 ratio, HPV-positive women	29%:71%[Chapter 6]		Multiplied with (*(1)* × (1 - *(4)*)) for strategy A or (*(1)* × *(2)* × (1 - *(5)*)) for strategies B and C, and the respective progression rates, it equals the proportion of women treated at follow-up in strategies B&C. Multiplied with the cost per CIN 0 or CIN 1 case, determines the total cost of referral of CIN 0/1 in strategies A, B and C.
(8) CIN 2 : CIN 3 ratio	75%:25%‡		Multiplied with *(3)*, *(4)*, or *(5)* (depending on the strategy), the recurrence rates after treatment, and the cost per CIN 2 or CIN 3 case, determines the total cost of referral of CIN 2/3 in all strategies.

t=0: at the time of the second BMD smear. t=6: six months after the second BMD smear. †Estimated by the multi-state Markov model from the Erasmus MC trial (see Material and Methods). ‡Not varied by HPV status, as observed in the Erasmus MC trial.

from the literature[104-109, 226] and used them as the basis for univariate sensitivity analyses. In all cases where independent observations were available, these formed an interval around the point estimates observed in the Erasmus MC trial (Table 7-1).

We defined the average duration of follow-up as the period following the second BMD smear in which women are further triaged through the HPV test (strategies A, B and C), referred and followed-up. We assumed compliance with the recommended follow-up (surveillance) after colposcopy. In the Netherlands, women with CIN 0/1 at initial referral are not treated but it is recommended to follow them up with 2 smears within 12 months; women with CIN 2/3 at initial referral are offered treatment and are then followed-up with 3 surveillance smears within 24 months.[230] If in either case at least one surveillance smear is abnormal, the woman is referred for colposcopy again and eventually (re-)treated. We accounted for the extra follow-up time due to surveillance based on the progression/persistence rates of CIN 0/1 observed in the Erasmus MC trial, and on post-treatment residual/recurrence rates for CIN 2/3 extracted from the literature (Table 7-2). In this way, we neglected the extra follow-up time because of negative colposcopies in women with (false-)positive surveillance smears. We assumed that the abnormal surveillance smear is found at the mid-point of the recommended follow-up interval. The assumed time needed between the successive management steps is then as follows: 2 months from the positive triage test to colposcopy, 1 month from colposcopy to treatment, 6 months for post-colposcopy surveillance of CIN 0/1, and 12 months for post-treatment surveillance of CIN 2/3.

We estimated that from the moment the referral advice is given it takes on average 10, 14, and 17 months to complete follow-up for CIN 0, CIN 1, and CIN 2/3, respectively. These estimates, used for the evaluation of the conventional strategy D, are based on the combination of the observed persistence/progression proportions (Table 7-2), and the recommended length of post-referral follow-up, both per CIN stage and regardless of the HPV status. In strategies A, B and C all referred women are HPV-positive. Untreated HPV-positive CIN 0/1 lesions are more likely to be referred once again due to higher persistence/progression proportions of the lesion than the HPV-negative CIN 0/1 lesions (Table 7-2). In these strategies, therefore, the estimated average follow-up time per CIN stage increases to 13 and 15 months for CIN 0 and CIN 1 lesions, respectively.

Direct medical costs, and the time and travel cost incurred by women were included in the analysis. The costs per procedure, and the average number of diagnostic and treatment procedures

Table 7-2. Proportion of women with persistence or progression of the initial lesion, as observed during 12 months in the Erasmus MC trial.

Initial CIN 0 lesion to >CIN 0†	14%
HPV-negative at enrolment	7%
HPV-positive at enrolment‡	30%
Initial CIN 1 lesion to ≥CIN 1†	38%
HPV-negative at enrolment	26%
HPV-positive at enrolment‡	48%
Post-treatment recurrent or residual lesions in CIN 2/3[j]	10%[232]

†Determined by pooling the trial outcomes for HPV-positive and HPV-negative women. Used for evaluation of strategy D. ‡Used for evaluation of strategies A, B and C. [j]Used for evaluation of all four strategies.

during post-referral management (diagnostics, treatment and follow-up) per CIN grade regardless of the HPV status, represent the recent Dutch situation[69, 231] (Tables 7-3 and 7-4). The average number of procedures per CIN stage[69] are based on the assumptions that (a) the given CIN grade is the maximum CIN grade, and that therefore women with CIN 0 are never treated, (b) 44% of women whose CIN 1 lesion is expected to persist or progress[37] are subsequently treated, (c) all women with CIN 2/3 are treated immediately, and (d) 10% of the women treated will need retreatment.[232] These assumptions and the estimated treatment modalities published earlier[41, 231] were validated against the most recent available individualised national data on diagnoses, diagnostic procedures and treatments.[128, 225] For the purpose of the present cost analysis, we also accounted for progression of CIN 0, and at least persistence of CIN 1 lesions by HPV status (see Table 7-2). We assumed that all women showing progression (or persistence of CIN 1) are treated.

7.4. Results

While all women undergo a colposcopy under the conventional strategy (strategy D), only 51%, 42% and 42% would have been triaged to it by HPV under strategies A, B, and C, respectively (Table 7-5). The expected total detection rate of women with high-grade CIN lesions (CIN 2/3) would be 18%, 18%, 18% and 19% of the eligible women under strategies A, B, C, and D, respectively. The avoided referrals would therefore predominantly concern women with at most low-grade CIN lesions (CIN 0/1). While 81% of all women with two consecutive BMD smears turned out to have CIN 0/1 at referral in the trial (strategy D), this would only be 33%, 23% and 23% under strategies A, B, and C, respectively. As a consequence, the total treatment proportion would amount to 32%, 28%, 28% and 41% under strategies A, B, C, and D, respectively. This proportion includes immediate treatment of women with CIN 2/3 lesions at referral, and later treatment of initially untreated women (CIN 0/1 at referral) who show abnormalities in follow-up.

We estimated that the total follow-up after the second BMD smear takes 13 months on average under the conventional strategy D (Table 7-5). This period predominantly reflects the time needed to complete the recommended management after referral. Therefore, the average length of follow-up per strategy is strongly affected by the lower referral rates in the HPV strategies. These outweigh the prolongation of the pre-referral period due to the extra HPV triage, so that in the end the completion of follow-up after the second BMD smear would on average take 8, 12, and 10 months under strategies A, B, and C, respectively. The expected average difference between strategies A and C is small though it should be noted that the subgroup that is referred based on delayed HPV testing (strategy C) is at a disadvantage. This is because they spend extra time in triage while the fact that they are referred and their post-referral management do not change.

We estimated that the total cost to manage a woman with two consecutive BMD smears under the conventional strategy (D) is €740 (Table 7-5). HPV testing itself would add to the triage costs, but these extra costs would be lower than the savings due to fewer referrals. The resulting difference is most favourable for strategy B (a decrease of 36% compared to strategy D), followed by strategies C (35%) and A (30%).

In the sensitivity analysis, we varied the epidemiologic assumptions with ranges from the literature reported in Table 7-1. In Table 7-6, we present the effects on the referral rate, the average length of follow-up and total costs. The ranking of strategies does not change. All three HPV strategies would remain more favourable than the conventional strategy. Under all investigated possibilities except when a lower HPV persistence rate is assumed (60% instead of 82%), strategy A would remain the most favourable in terms of the time needed to complete the total recommended follow-up. When the lower HPV persistence rate is assumed, strategy C could decrease the average time in follow-up to the same level as strategy A. The proportion of women referred and treated, as well as the total cost, would remain the lowest under strategies B and C: the referral rate in the range of 31-49%, and the cost decrease compared to strategy D in the range of 33-51%. Our results are most affected by the changes in the assumptions on HPV prevalence and its persistence within 6 months (direct observations for the latter are not available). This is not surprising since these determine how many women will be ultimately referred for colposcopy.

Table 7-3. Average number of diagnostic and treatment procedures per CIN grade regardless of HPV status, based on national data for the Netherlands.[69]

	Histology			
	CIN 0	**CIN 1**	**CIN 2**	**CIN 3**
Diagnostic procedures:				
Colposcopy	2.11	4.45	4.77	5.05
Smear by a gynaecologist	1.61	2.59	3.10	3.13
Biopsy	0.37	0.63	0.65	0.62
Endocervical curretage	0.13	0.37	0.35	0.38
Smear by a GP	0.40	0.51	0.44	0.32
Treatment procedures:				
LETZ†	0.13	0.42	0.93	0.75
Conization	0.01	0.04	0.15	0.30
Hysterectomy	0.00	0.02	0.02	0.04

†Loop excision of the transformation zone.

7.5. Discussion

Our analyses showed that compared to direct referral of women with two consecutive BMD smears to colposcopy a referral based on HPV testing can prevent at least one out of two colposcopies and treatment in one of three women with at most low-grade lesions. It can decrease the average follow-up time by half a year, and reduce the associated average total costs by a third. In the population of 3.4 million women at risk (i.e., with a cervix) aged 30-60 in the Netherlands, around 8,700 annually have a BMD primary screening programme smear in the screening programme, of whom around 2,200 are referred due to BMD persistence.[225] Even if strategy A with the lowest expected cost decrease would be adopted instead of the currently recommended direct colposcopy (strategy D), total annual savings of close to €0.5 million could be attainable in this group of women in the Netherlands. BMD primary smears outside of the screening programme account for roughly half of the BMD primary smears in the Netherlands,[225] so savings could double if the recommendation for HPV triage would extend from smears within the screening programme to all smears. Savings per screened woman could be higher in areas where the proportion of BMD primary smears is higher (e.g. >5% in Finland and England compared to <2% in the Netherlands).[Chapter 2]

None of the three analysed HPV strategies is optimal for both side effects and costs. Delaying the HPV testing by 6 months (strategy B) may have the lowest total costs (Δ=-36% compared to direct colposcopy in strategy D) but for the combination strategy C the costs are only slightly higher. The risk selection is equally good for strategies B and C (both strategies establish HPV persistence before a referral), but the period of time in which women are kept in triage is on average 3 months shorter for strategy C. Strategy A eliminates the need for an extra triage period and extra GP visits, but it is less powerful in selecting women at higher risk for progression to cancer than strategies B and C.

The rationale for screening programmes is early detection and treatment of disease. In our analyses, we assumed equal effectiveness of each triage strategy, i.e. that there are not more cervical cancer deaths in the HPV strategies than in the direct colposcopy. This assumption can be challenged for two reasons. First, in the HPV strategies only women with detectable HPV infections, i.e. women at risk for cancer, are referred for colposcopy. A recent meta-analysis estimated that 95.5% of all CIN 2+ lesions can be identified if women with primary ASCUS and LSIL smears (which approximately correspond to BMD smears) are tested for HPV.[233] Because at the time of histologic testing some women may have already cleared the HPV without yet having their lesion regress, or might still clear the HPV later, only (an unknown) part of the remaining 4.5% may represent potential loss of sensitivity in detecting CIN 2+ compared to direct colposcopy (strategy D). On the other hand, recent data from the ALTS trial suggests that HPV testing may perform no worse than, or may even outperform colposcopy in identifying high-grade CIN lesions.[234] Follow-up data from the currently on-going randomised trials, e.g. that from the POBASCAM study expected shortly,[15] will shed more light on the loss of sensitivity of HPV triage due to less frequent referral. Second, in the Netherlands the conventional guidelines of following up the BMD primary smear by a follow-up smear in 6 months implicitly accept the risk of postponing treatment to occult underlying cancers in 0.4 per 1,000 women with BMD primary screening smears.[225] When HPV testing is delayed for another 6 months (i.e., to 12 months after the BMD primary smear), the risk of diagnosing an invasive cancer is a further 0.9 case per 1,000 women with a BMD primary screening smear.[225] Should strategies B or C be adopted, postponing referral would miss another six months of lead-time for treatment in these 0.9/1,000 women. Given that these are screen-detected cancers, one may assume that despite this delay it is likely that they are still found at an early enough stage of invasion to retain the good 5-year prognosis of 90% survival.[235] To sum up, we can reasonably assume that the effectiveness of the three analysed HPV triage strategies and of the conventional strategy are comparable.

An alternative to the analysed triage strategies could be to drop the requirement of first establishing the persistence of the BMD smear and to instead triage solely through HPV, that is directly after one primary BMD smear. Per 1,000 women with primary BMD smears, such an approach would expectedly increase the referral rate by 100, the CIN 2/3 detection rate by 10-15, and prevent 1-3.5 cancers (Appendix, Chapter 7.6.). Given the very long (>10years) average duration of preinvasive lesions,[34] some of these 1-3.5 per 1,000 women will still have the chance to be managed early at the next screening round in less than 5 years. This balance is not straightforward, and remains uncertain. Again, the expected long-term follow-up data from

Table 7-4. Unit costs (€ 2005) of medical procedures, visits and hospital stays, based on the recent Dutch data.[69, 231]

Procedure	Unit cost
Pap smear, taken by a GP†	45
Pap smear, taken by a gynaecologist‡	25
Co-collected HPV test	1
HPV test at an extra GP appointment∫	59
First colposcopy	86
Second or later colposcopy	63
Biopsy	50
Endocervical curettage	81
LETZ§	490
Conization§	1,195
Hysterectomy§	4,176
Average total cost (referral + post-referral management):	
- CIN 0	336 (420 if HPV-positive)
- CIN 1	828 (884 if HPV-positive)
- CIN 2	1,239
- CIN 3	1,432

†Includes the visit at the GP and the collection of sample material (€21), laboratory cost (€17) and costs of the woman (€6). ‡The laboratory cost. Collection of sample material and costs of the woman are included in the cost of colposcopy. ∫Includes visit at the GP and collection of sample material (€21), laboratory costs (€33), and costs of the woman (€6). §Treatment costs include the charge per type of treatment (LETZ €294, conization €477, hysterectomy €1,062), cost of outpatient visit (if the procedure is performed in an outpatient setting; €64), cost of hospital days (day care €229, hospital day €359), preoperative diagnostics (for conization or hysterectomy €98) and costs for the woman (€9 for an outpatient visit, and €42 per treatment day).

Table 7-5. Results: Number of procedures, side effects, and costs (€ 2005) due to HPV triage, diagnostic assessment and treatment per strategy, and per woman with two consecutive BMD smears. Base case assumptions.

	Triage strategy			
	A (Immediate triage)	B (Delayed triage)	C (Two-stage triage)	D (Direct colposcopy)
Number of procedures, side effects				
HPV tests – co-collected samples†	4.00	0.00	4.00	n.a.
HPV tests – samples collected at extra GP visits‡	0.00	1.00	0.51	n.a.
Proportion of women referred∫	51%	42%	42%	100%
Detection of CIN 0§	10%	7%	7%	36%
Detection of CIN 1§	24%	17%	17%	45%
Detection of CIN 2§	13%	14%	14%	14%
Detection of CIN 3§	4%	5%	5%	5%
Proportion of women treated∞	32%	28%	28%	41%
Average time in follow-up (months)¶	7.7	12.4	9.5	12.9
Costs				
HPV tests	37	59	67	n.a.
Referrals to colposcopy	478	412	412	740
Total	515	472	479	740
HPV tests (extra GP visits)				
Per avoided referral	0.0	1.7	0.9	n.a.
Per avoided treatment	0.0	7.8	4.0	n.a.

t=0: immediately after the second BMD smear. t=6: six months after the second BMD smear. n.a.=not applicable. †E.g. for strategy C: It is observed in the Netherlands that 25% of all BMD primary smears have a repeat BMD smear.[225] This means that for every woman with the second BMD smear, 4 co-collected HPV samples need to be taken in total. ‡E.g. for strategy C: 51% of women with two BMD smears are HPV-positive at t=0, and are retested for HPV at t=6. ∫E.g. for strategy C: 51% × 82% HPV persistence rate (Table 7-1). §Directly observed in the Erasmus MC trial for strategies A and D (see Table 7-1), and estimated for strategies B and C. ∞E.g. for strategy C: women with HPV-positive CIN 2/3 (18%, see Table 7-5) + persistence or progression in women with initial CIN 0/1 lesions (7% × 30% + 17% × 48%, see Tables 7-2 and 7-5). ¶E.g. for strategy C: 51% HPV-positive at t=0 × 6 months in triage + post-triage follow-up (7% × 13 months for CIN 0 + 17% × 15 months for CIN 1 + (14% + 5%) × 17 months for CIN 2/3, see Table 7-5 & Methods).

randomised trials such as the POBASCAM study should in the near future help improve these estimates. Also, adding further markers to HPV-testing (e.g. typing for HPV 16 and 18, testing for mRNA) is currently under investigation as feasible ways to improve specificity, which would more likely favour HPV-testing above cytological triage.

This study has some limitations. First, some women who in the Erasmus MC trial had a persistent HPV-positive CIN 2/3 lesion from t=0 to t=6 (this prompted treatment and censoring from further follow-up) could have cleared the HPV by t=12. Therefore the assumption we used to fit our interpolation model with – that women treated at t=6 would have remained in the same state until t=12 had they not been treated – may give an overestimate of HPV and CIN 2/3 persistence, and an overestimate of the referral rate and costs for strategies B and C. On the other hand, the quantification of strategies B and C is based on the data from a trial in which biopsies were taken at enrolment from all colposcopic abnormalities. Biopsies may have interfered with the development of the disease, e.g. by removing HPV-infected lesions which would not have cleared and/or regressed if left unbiopsied in the observed period. Consequently, the estimated 6-month HPV persistence rate may be too low, the CIN 2/3 prevalence rate at t=6 too low, and the expected decrease in the referral and total costs too high. Though especially strategy C seems interesting for implementation, we are less certain exactly how well it would in reality perform relative to strategy A. Given our assumptions, strategy C could avoid a referral to colposcopy in at most 9% more women than strategy A. The cost for this advantage is an extra GP visit and a 6-month longer triage period for 51% of women in strategy C. In the base-case calculation the extra GP visits in strategy C save 0.18 referrals and 0.07 treatments compared to strategy A. Strategy C would be preferred over strategy A only when it could be shown that the perceived burden of an extra GP visit and 6-month waiting time during triage will be less than 18% of that associated with a referral for colposcopy, and also less than 7% of that of treatment. The relative burden is thus far unknown but it could be studied in an implementation trial in which the 6-month natural history of HPV could be monitored for this group of women without early biopsy interference.

Second, because we wanted to study the optimal strategy to offer to women, we assumed compliance with follow-up in

Table 7-6. Results: Triage outcomes (referral rate, length of follow-up) after changes in assumptions on HPV prevalence and persistence, and CIN 2/3 prevalence. Sensitivity analysis.

	Strategy A			Strategy B			Strategy C			Strategy D		
	% Referred	Follow-up (months)	Total cost	% Referred	Follow-up (months)	Total cost	% Referred	Follow-up (months)	Total cost	% Referred	Follow-up (months)	Total cost
Baseline assumptions	51%	7.7	515	42%	12.4	472	42%	9.5	479	100%	12.9	740
Lower HPV prevalence†	40%	6.1	412	33%	11.0	383	33%	7.4	384	100%	12.9	740
Higher HPV prevalence†	60%	9.1	599	49%	13.5	544	49%	11.1	557	100%	12.9	740
Lower HPV persistence‡	51%	7.7	515	31%	10.7	361	31%	7.8	369	100%	12.9	740
Higher HPV persistence‡	51%	7.7	515	43%	12.6	487	43%	9.7	494	100%	12.9	740
Lower CIN 2/3 prevalenceʃ	51%	7.6	474	42%	12.3	440	42%	9.4	448	100%	12.6	679
Higher CIN 2/3 prevalenceʃ	51%	7.9	556	42%	12.6	508	42%	9.6	515	100%	13.9	882

†Baseline assumption: 51%. Range: 40%-60%. ‡Baseline assumption: 82%. Range: 60%-85%. ʃBaseline assumption: 19% (all at t=0), 35% (HPV-positive at t=0), and 44% (HPV-positive at t=6). Range: 10%, 20%, 30% (low), 40%, 50%, 60% (high).

all strategies during the triage period. Lack of follow-up will decrease screening effectiveness. It has been shown in the literature that up to one third of the women do not comply with follow-up,[236] and that the compliance decreases with longer time lags in recommended follow-up.[237] Since women in our study have already had to wait for 6 months for the follow-up smear, these findings are especially challenging for those strategies that involve extra prolonged periods of triage in which HPV is allowed to clear (strategies B and C). In the Netherlands, cervical cancer screening is performed at the primary health care level by general practitioners (GP). A large audit of GP practices in the Netherlands has shown that complex decision trees are important barriers to compliance of the GP with diagnostic guidelines.[143] All three HPV strategies increase the diagnostic complexity for the GP as they add to the currently routine practice a test of a different type, strategies B and C also at extra time points.

Third, women's preferences should play a role in optimising the triage strategies. In principle, triage through HPV could decrease anxiety in women with abnormal smears by decreasing the number of false-positive (i.e., HPV-negative) referrals. Still, follow-up of a large UK screening cohort showed that a negative HPV result does not significantly reassure women with a BMD smear.[238] Moreover, women who received a positive HPV report showed even higher anxiety levels after an abnormal smear.[239] Several surveys have shown that a significant proportion of women with cytologic abnormalities may prefer an early referral to wait-and-see approaches,[240, 241] and that a higher level of psychological distress in a woman is an important factor contributing to such a choice.[242] It seems then that it is especially the postponement of action in women who know that they tested HPV-positive in strategy C that could negatively affect their well-being, making strategy A more appealing to implement.

In conclusion, our analysis provides further evidence that HPV can improve the specificity of referral for colposcopy of women with persistent BMD smears, and decrease the burden on both the women and the health-care system. Given the established high sensitivity of HPV testing for progressive cervical neoplasia, we therefore advocate including HPV testing before referring women. For these women, who are already in follow-up for 6 months, analysis of additional aspects favours implementation of immediate HPV testing without waiting another 6 months for clearance.

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7.6. Appendix: Expected referral and detection rates for HPV triage, with and without cytology in triage

Assume strategies A1, B1 and C1 such that relative to the primary BMD smear (found at t=-6) the timing of HPV testing remains equal as in strategies A, B and C but the cytologic triage (at t=0) is not done. As a consequence, HPV testing is done on all women with a BMD primary smear in strategies A1, B1 and C1, whereas it is only done on women with two consecutive BMD smears in strategies A, B and C.

The expected referral rates, expressed as the proportion of all women with a BMD primary smear, are:

- Strategy A: 18% (25% with BMD in follow-up[225] × 51% HPV-positive[Chapter 6] + 5% with >BMD in follow-up;[225] Figure 7-A1);
- Strategies B and C: 15% (25%[225] × 51%[Chapter 6] × 77% (Table 7-1) + 5%[225]);
- Strategy A1: 28% (35% of the women with a BMD primary smear are HPV positive[15] × 81% HPV persistence rate in 6 months;[243] Figure 7-A1); and
- Strategies B1 and C1: 24-28% (35%[15] × [68% (18 months)[243] to 81% (6 months) HPV persistence rate[243]]).

The expected increase in the detection rate of CIN 2/3 lesions in strategies A1, B1 and C1 over those of strategies A, B and C is 13.5 per 1,000 women with a BMD primary smear ((95% - 80%) difference in sensitivity for CIN 2+ of HPV vs. cytology triage[233] × 9% prevalence of CIN 2/3 lesions in BMD primary smears[109] × 1,000).

It has been estimated that 10-24% CIN 2/3 lesions eventually progress to cancer.[32, 37] Therefore, substituting the combination of a follow-up smear and an HPV test for a stand-alone HPV test in women with BMD primary smears could prevent at most 1.4 to 3.2 ([10% to 24%] × 13.5) invasive cancers.

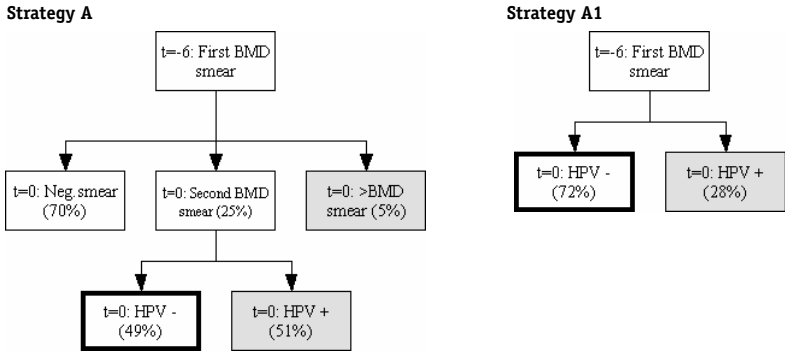


Figure 7-A1. Calculation of expected referral rate in strategies A and A1, after a single BMD primary smear. Shaded rectangles represent women who need to be referred, whereas the rectangles with a bold border represent women for whom we assumed that they return to the regular screening programme.

CHAPTER 8. General Discussion

In this thesis, we studied the recent developments in the Dutch cervical cancer screening programme. In the Netherlands, cervical cancer incidence and mortality have been decreasing.[4, 23, 24] At present, cervical cancer is not anymore a common cause of death.[4] Even though mortality already decreased before screening became widespread, the continuing decrease in the past three decades was also due to the screening programme: it was estimated that with a 75% coverage, the risk of dying from cervical cancer would decrease by 50%.[163] Women in a wide age range 30 to 60 years are invited every 5 years to have a Pap smear taken within the screening programme for which at present about €30 million are spent yearly.[60]

In this thesis, we showed that after the changes in the programme's organizational and financial protocols and in medical guidelines since 1996, the extent of screening-induced side-effects decreased considerably without negatively influencing the programme's potential to prevent cervical cancer mortality. The estimated cost per life-year gained through screening, €9,000 (3% discounting of costs and effects), is acceptable.[69] Further, our research on HPV testing showed that the screening interval could be safely prolonged to more than 5 years if HPV test would replace the Pap smear, and that adding the HPV test to the triage of women with persistent low-grade cytological abnormalities can considerably decrease the present costs and side effects of follow-up.

8.1. Answers to research questions

In this chapter, we will formulate and discuss the answers to the research questions that we wanted to address.

1. What were the short-term effects of changes in the screening programme protocols and guidelines since 1996?

The screening coverage in the target age group increased, primarily due to the substantial increase in the added age groups (30-34, and 55-64 years). The proportion of women with a non-negative smear decreased by 80%, more women were compliant with the follow-up advice, and follow-up was completed faster. The total number of smears dropped by 20%, principally due to fewer follow-up smears. Overall, the changes in the programme since 1996 led to an increase in the screening coverage, and a decrease in the screening-induced negative side effects.

Chapter 2 of this thesis took two snapshots of the screening programme, one in 1994 and another in 2003. In the meantime, more recent data (until 31st March 2007) became available. This data shows that, first, improvements in the process indicators (the participation rates and the screening coverage, the proportion of women with positive results, the compliance with follow-up recommendations, the number of smears made) were seen within 3 to 5 years after the implementation of the new protocols and guidelines, and second, that most process indicators stabilized thereafter (Appendix, Chapter 8.6.).

Since 1996, the coverage increased substantially,[Chapter 2] but even with these high coverage rates, the majority of women diagnosed with cervical cancer were not screened, or were not screened regularly.[72] This stresses the importance of further increasing the coverage. Several recent and on-going trials in the Netherlands are exploring new instruments to increase the coverage rate, e.g. self-sampling for HPV.[131, 244] While the coverage rates have improved predominantly for older women (55-64 years), no improvement has been observed since 1998 among women aged 30-34 years. For these women, the coverage rates are still lagging behind those of older women by about 10 percentage points. One of the tentative explanations for the relatively low coverage rate is that around 13-14% of women aged 30-34 were pregnant at any point in time in the period 2000-2006.[245] Pregnancy is a reason for delaying the smear (since 2006 not also during the lactation period).[57, 58] A more targeted scheduling of screening reminders for women who notify the screening organisation or their GP that they are pregnant could be one of the coverage-increasing options.

The 1996 change discouraging screening before the age of 30 gives new insights into whether the starting age under 30 years would be beneficial. Before 1996, more than one-quarter of women aged 20-29 had at least one smear in the preceding 5 years, whereas by 2001 the coverage rate dropped to less than 10%. Despite this, the incidence and mortality in age groups <30 and 30-34 did not increase.[246] Within 20 years a decrease should be observable in these age groups if in the coming years a considerable proportion of 12-year old girls will be vaccinated for HPV.

A lower cytologic threshold for referral to colposcopy of women with primary low-grade abnormal smears[Chapter 2] raised the fear that the demand for colposcopy would increase substantially. This was not the case (Table 8-1). Immediately after the introduction of the new guidelines, the referral rate indeed doubled. This can be explained by a combination of several factors: an increased number of (primary) smears in the first two years, an immediately increased proportion of primary smears with an immediate referral advice, and in the first 2 years a still high proportion of smears with low-grade abnormalities (Appendix, Chapter 8.6.). In the years that followed, however, the demand for colposcopy decreased and remained at the level similar to that before 1996.

2. Did the sensitivity of the Pap smear decrease after a broader definition of a negative smear?

No. The incidence of cervical cancer after a negative smear remained the same as it was before 1996, suggesting that the reduced smear positivity rate did not lead to a measurable decrease in smear sensitivity.

Interval cancers arise either because of fast-growing lesions, or because the preceding screening rounds missed the lesion. In the recent period, approximately one quarter of cancer cases per year were preceded by normal smears,[93] i.e. could be seen as interval cancers. These are the cancers that could theoretically be prevented by a more sensitive screening test, e.g. an HPV test. However, the proportion of cases preceded by a negative screening test depends on the screening coverage. For example, in a hypothetical situation with 100% coverage, all cancers after

Table 8-1. The estimated number of women referred to a gynaecologist due to cervical abnormalities (directly or after positive triage of low-grade abnormal smears), per 100,000 women at risk, per year.[23, 93, 128]

	Estimated number of women referred (×1,000)†	Population at risk aged 20-84 (×1,000)	Estimated number of women referred per 100,000 population at risk
Year	**A**	**B**	**C=A/B**
1990	8	5,097	152
1991	8	5,150	148
1992	8	5,199	149
1993	7	5,237	142
1994	8	5,269	150
1995	7	5,288	135
1996	16	5,307	299
1997	12	5,328	231
1998	9	5,354	170
1999	8	5,385	145
2000	7	5,420	128
2001	7	5,459	129
2002	7	5,496	132

†Regardless of the reason for smear-taking. Including women with a direct referral, positive triage of low-grade abnormal smears, or early referral.

the first screening round would be interval cancers. It can therefore be expected that it if the coverage will continue to increase, the proportion of interval cancers will also increase.

For effective screening, the management of positive screening results needs to be successful, as well. This is pursued by cytological follow-up, or by CIN treatment and post-treatment cytology. Negative follow-up (2 consecutive negative smears after a BMD smear, and 3 consecutive negative smears after CIN treatment) is seen as sufficient evidence of absence of clinically relevant lesions. Like women with negative screening results, women with negative follow-up are assumed to be at low risk for cervical cancer and are recommended to continue participating in regular screening. This assumption remains to be empirically tested, both for BMD smears as well as for CIN lesions.

3. Can screening be ceased for women with several negative smears by age 50?

The risk for cervical cancer after the third consecutive negative smear around age 50 is comparable to the risk in younger women with similar screening histories. This does not speak in favour of making the screening policy less intensive for these older women but not similar younger women.

If surrogate endpoints are improperly used, they may lead to wrong conclusions.[247] Also in our study, the CIN detection among well-screened women around age 50 was much lower than in younger women. In this respect, it corroborated the results of other studies. There is, however, strong evidence that the progression of CIN to cancer increases with age,[34] and our study showed that the conclusions based on cervical cancer, the true clinical endpoint, differ from those obtained from CIN. Therefore, this analysis is an example of where CIN as a surrogate endpoint leads to the wrong conclusion, and underscores that its use should be carefully evaluated.

The 1993 *ex-ante* cost-effectiveness analysis concluded that for the Netherlands the most efficient cervical cancer screening policies would end only around age 70, almost irrespective of the screening interval (range: 5-10 years).[55] Unless a new HPV vaccine will be developed which will be effective in women who were previously infected with HPV, screening will for at least four decades remain the only available option for cervical cancer prevention in older women. Still, while our study suggested that it is just as worthwhile to continue to screen older as younger women after several negative smears, it showed that, in fact, the incidence of cancer among both is low. Further research should clarify whether women with well-documented adequate and negative screening histories could be offered less intensive screening schedules.

4. What is the negative predictive value of the HPV test compared to the Pap smear in primary screening?

In a European study, the incidence of CIN 3+ six years after a negative HPV test was lower than three years after a negative smear. If in the Netherlands the HPV test would be used in primary screening, the screening interval could be safely prolonged to more than the current 5 years.

These results are in line with the published long-term outcomes of randomized trials from the Netherlands[98] and Sweden.[99] A longer screening interval could provide some counterweight to the (currently still) higher price of the HPV test compared to the Pap smear, and to an expected increase in follow-up testing. The latter may occur because the HPV-positivity rates are generally higher than the smear positivity rates (by a factor 2 to 4).[98, 99]

Any new increase in test positivity would have to be balanced with the public health benefit measured in a lower incidence of cervical cancer in women with a negative HPV test than it is presently the case. This data is not yet available. The individual trials pooled into the European study, as well as the currently on-going randomised trials were powered to high grade CIN (CIN 2+ or CIN 3+) as the endpoint. This was recommended as the best feasible endpoint for establishing the efficacy of

new cervical cancer prevention technologies, i.e. HPV testing and HPV vaccination.[248] For modelling studies based on this data, extrapolation from CIN to cancer will need to be made. To support such extrapolation, the relative risk of cytology-negative HPV-positive CIN, i.e. of CIN that would be missed by cytological but not HPV screening, should be established. Invasive cancer (the true endpoint), on the other hand, would require much larger studies. Instead, pooling the data from the randomised trials[46] once it becomes available will allow an unbiased estimate of the increase in the programme sensitivity from using the HPV test.

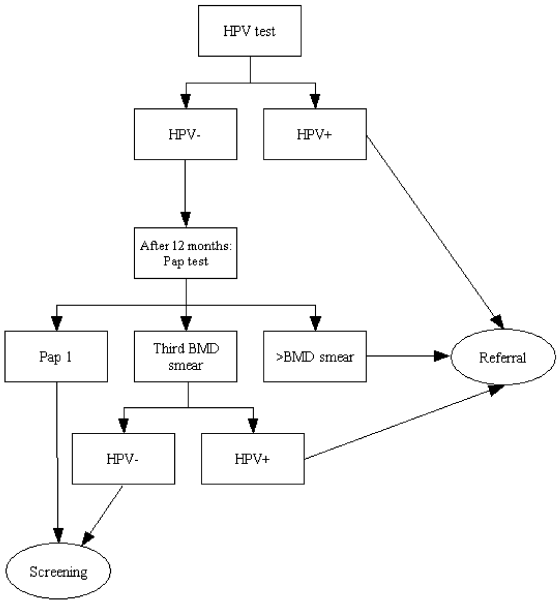
5. What is the optimal use of HPV testing in triage of women with a persistent low-grade abnormal smear?

By restricting gynaecologic follow-up to the HPV-positive women with a persistent low-grade abnormal (borderline or mildly dyskaryotic) smear, gynaecologic follow-up in the HPV-negative women is not needed anymore, and prevention of cervical cancer will not be compromised.

For women with a persistent low-grade abnormal smear, the 2006 Dutch pathology guidelines recommend immediate HPV testing, and an immediate referral to a gynaecologist in case the HPV test is positive (Figure 8-1).[58] However, according to these guidelines women with a negative HPV test should undergo a second combined follow-up test (cytology + HPV) 12 months later. For these women, the final decision on whether they can rejoin regular screening or need to be referred, will be made only at this point. The complexity of such a follow-up process is considerable. In the Erasmus MC trial, almost all of the initially HPV-negative women could rejoin regular screening after 12 months.[Chapter 6] Moreover, an HPV-positive test 12 months after an initial HPV-negative test might merely signal a new HPV infection associated with a new cervical lesion that will, if at all, only progress to cancer in many years.[32-34] Nevertheless, a small residual risk may exist. In this context, the next regular screening round could serve as a safety net, at least for women aged ≤55 years who will still be invited to screening.

The impression is, therefore, that the pathology guidelines from 2006 are more geared towards protecting the sensitivity than the specificity of the programme. Due to the relatively small study size and the relatively short follow-up time in the Erasmus MC trial we could not analyse the (relative) effectiveness of all strategies. Several well-documented factors, e.g. the long preinvasive period,[32-34] and the higher than 90% sensitivity of HPV for detecting underlying high-grade CIN lesions in low-grade abnormal smears[208, 233] support the view that following-up women who tested HPV-negative may not bring about considerable clinically relevant benefits. This, however, remains to be confirmed by the long-term follow-up studies from randomised trials.[46]

New (post-2006) guidelines (2-stage HPV testing)



Strategy proposed in Chapter 8 (1-stage HPV testing)

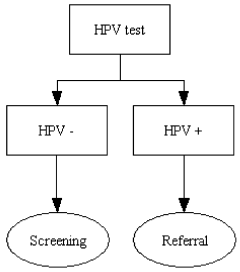


Figure 8-1. The new (post-2006)[58] pathology guidelines for management of women with a second low-grade abnormal smear, and the strategy proposed in Chapter 8.

8.2. The reliability of the PALEBA/PALGA data

In the past decade, the PALEBA/PALGA data has been used as the main data source for the national evaluation of the Dutch cervical cancer screening programme. Upon retrieval from PALGA, the registered information is translated into PALEBA/PALGA categories. This is done in order to decrease the complexity of the PALGA data in the case of test outcomes, or to make an inference on the likely sequence of the tests, which is not directly registered in PALGA. This process is described in detail elsewhere.[83] In order to get insight into how reliable PALEBA/PALGA is, we compared it with two other data sources: with the data from the Dutch Cancer Registry,[4] and the data obtained from the pathology laboratories of one of the screening regions (Region West: the SBBW data, made available by the Leiden Cytologic and Pathologic Laboratory).

Table 8-2. The number of incident invasive cancers in PALEBA/PALGA, and in the Dutch Comprehensive Cancer Registries, by year and age at diagnosis.[4, 93]

Year	Age group											
	<30		30-44		45-59		60-74		≥75		Total	
	P	CR	P	CR	P	CR	P	CR	P	CR	P	CR
1994	37	24	271	257	203	180	166	146	101	109	778	716
1995	43	44	313	293	192	170	156	135	85	85	789	727
1996	36	32	333	286	182	166	145	138	96	98	792	720
1997	42	36	322	279	200	180	153	149	96	91	813	735
1998	38	42	272	278	180	176	142	150	85	103	717	749
1999	35	39	295	290	187	170	125	114	89	90	731	703
2000	22	15	279	265	187	169	140	123	78	108	706	680
2001	28	27	258	244	173	157	111	94	78	82	648	604
2002	33	29	250	234	185	168	135	127	86	86	689	644
2003	24	21	249	223	176	157	105	82	97	101	651	584
PALEBA vs. CR†	109	100	107	100	110	100	110	100	93	100	107	100

P = PALEBA/PALGA, CR: Dutch Comprehensive Cancer Registries. †CR=100. Average for years 1994-2003.

Comparison with the Dutch Cancer Registry data

At present, PALGA is the only national data source relating the individual cervical cancer cases with their screening histories. Since cancer is principally a pathological diagnosis, most cases are registered in PALGA. Only a minority of the cancer cases (less than 5%)[249] is not based on pathology. PALGA is (one of) the main sources of data for the Dutch Cancer Registry (CR). The latter is authorised to also access e.g. the individual hospital patient data, and is this way able to verify the individual PALGA diagnoses. At present, however, there is no link back to PALGA with cases accepted as cancer cases in the CR.

For PALEBA/PALGA, complete histories (including the pathologist's free-text specimen descriptions) were retrieved for women with at least suspected cervical cancer registered among the Thesaurus-like codes since 1994. The final PALEBA/PALGA diagnosis of cervical cancer was based on the best judgement of all the codes and the free-text entries for histology. In the period 1994-2003 (the last year for which the CR data is currently available), PALEBA/PALGA contains on average 7% more incident cancers than CR: about 10% more up to age 74, and 7% fewer at older ages (Table 8-2). By calendar year, this gap was neither systematically decreasing nor increasing. Thus, while the incidence rates in PALEBA/PALGA by age 74 are somewhat too high, the conclusions from analyses presented in this thesis (a comparison between two calendar periods in Chapter 3, and a comparison between the age groups in Chapter 4) were not affected.

Comparison with the laboratory data from Region West (SBBW)

In the SBBW data, covering The Hague, Leiden, Delft, Gouda, and the countryside between Rotterdam, Utrecht, Haarlem and the North Sea, the size of the target population is based on the actual number of invitations sent. The cytology reports of all programme smears taken from women in the postal area code of the SBBW are sent to SBBW from the 6 regional laboratories. In order to obtain information on histological follow-up, the SBBW sent the records of women with a follow-up advice to the pathology laboratories, and received structured follow-up data including pathology reports. In part, the pathologists also use the PALGA system for the retrieval of pathology diagnoses, e.g. if women are treated in hospitals outside of the region. Because all information can be verified, it is estimated that there are, unlike in PALEBA/PALGA, virtually no incorrect matches between primary screening and the follow-up data.

We compared the two datasets based on several process indicators for the programme in 2003 (Table 8-3). The difference in most screening process indicators (the size of the target population, the proportion of positive results, the repeat smear compliance and the detection rate of CIN 3+) was very small. This indirectly suggests that excluding women with the 0.5% most common surnames, as is done in all PALEBA/PALGA studies of follow-up, adequately avoids the problem of administrative twins. On the other hand, the number of primary programme smears in PALEBA/PALGA was 7% lower, and the detection rate of CIN 1 was 54% higher.

The lower number of programme smears in PALEBA/PALGA compared to the SBBW data was due to the data from one of the regional laboratories not being registered in PALGA. The missing data is going to be retroactively restored in PALGA, and will consequently be retrievable for PALEBA/PALGA.

The reason for the different estimate of the detection rate of CIN 1 is more complex. In PALGA, the (histological) diagnoses are registered with Thesaurus-compatible codes. Until recently, preinvasive findings could be described with a large number of codes. For PALEBA/PALGA, these have to be interpreted (translated) into CIN diagnoses. Compared with the pathology practice, PALEBA/PALGA seems to use a broader interpretation of CIN 1, one which includes codes of atypia, hyperplasia, dysplasia NOS with or without clear resection margins, etc. This assures that the smears made shortly after such a diagnosis would be interpreted as follow-up rather than primary screening smears, as it is more likely that these women remained under follow-up by a gynaecologist than rejoined the regular screening schedule. In order to avoid the problems of interpretation, PALGA in 2007 restricted the number of Thesaurus-compatible codes that can describe a CIN lesion. It is expected that as a consequence the detection rates from PALEBA/PALGA will decrease and/or those from other sources increase, thereby limiting the present

Table 8-3. Women invited to screening in 2003: Comparison of the SBBW and PALEBA/PALGA data for region West.

	SBBW data	PALEBA/PALGA data for region West[93]
Target population	81,555	81,768
Primary program smears	54,931	50,947
Attendance rate	69%	62%
Repeat smear advice†	1.89%	1.93%
Repeat smear compliance	85%	86%‡
Immediate referral advice	0.35%	0.33%‡
Histoscore: CIN 1	0.07%	0.11%‡
Histoscore: CIN 3+	0.31%	0.30%‡

†Due to Pap 2/3a1, or inadequate quality. ‡Excluding women with 0.5% most common surnames.

differences. The actual effects of the change in the registration should be observable already on short term.

Regional vs. national data: advantages and disadvantages

Evaluation of the Dutch cervical cancer screening programme takes place on the national and the regional level. These two levels are complementary. At the regional level, the data on the screening programme can be obtained directly from the laboratories, whereas additional information on e.g. follow-up can be obtained through linkage with PALGA. Strong points of regional monitoring are that it is easier to verify the final diagnoses, and that it gives the possibility of benchmarking and providing feedback to individual laboratories. On the other hand, it misses the data necessary for the evaluation of long-term screening effectiveness. For example, at the regional level it is at present not possible to evaluate non-programme screening, whereas in PALEBA/PALGA it is. Next, women may move across the borders of a single region, which makes it more difficult to determine their lifetime screening histories. Also, regional databases are underpowered to provide reliable frequency estimates of rare events, e.g. the interval cancers.

8.3. Other techniques and tools for cervical cancer prevention

The Pap smear helped reduce cervical cancer mortality,[43] but it is not perfect.[250] The efforts to improve it, and to improve cervical cancer prevention in general, have led to several other techniques and tools described below.

Liquid-based (thinlayer, monolayer) cytology, and automated screening

Using a conventional Pap smear, a sample of exfoliated cells from the cervix uteri is transferred and smeared onto a glass and fixed in order to prevent air-drying which would distort cellular detail.[251] In this process, errors may occur which may cause the smear to be unusable or suboptimal for evaluation. This is reduced with liquid-based cytology (LBC; marketed as e.g. ThinPrep, AutocytePrep, CytoRich, Cytoscreen, Papspin), in which the exfoliated cells are instead rinsed into a vial of fixative fluid.[251] LBC is a standard cytological primary test in the USA and England,[141, 252] where as a consequence the proportion of inadequate cytology tests recently halved from almost 10% to less than 5%.[253] At present, the proportion of inadequate quality smears in the Netherlands is low (about 1%), and LBC was not considered cost-effective for the introduction into the Dutch cervical cancer screening programme.[142] Even though it is not recommended within the screening programme, the use of LBC has nevertheless increased in the Netherlands.

At present, the cost of LBC is higher than that of conventional cytology.[231] Several studies claimed that this could be balanced by a decreased proportion of inadequate samples, and increased sensitivity and/or specificity. Two recent systematic reviews, however, could not substantiate these claims as they found no evidence that LBC performs substantially better than the conventional Pap smear.[254, 255] The average rate of inadequate samples was on average only marginally lower.[255] The LBC tended to increase the rate of low-grade abnormal cytologic results (ASCUS and LSIL).[255] No significant improvement in the detection of high-grade CIN lesions could be seen.[254]

Smears prepared for evaluation are read in pathology laboratories. During this labour-intensive process reading errors causing false-negative results may occur. This has been sought to be improved on by automating the reading of smears (e.g. the PAPNET, AutoCyte SCREEN, AutoPap 300QC, FocalPoint systems). In automated reading of smears, a computer system can either separate negative smears not needing manual reading from those that do deserve a cytotechnician's attention, or select the abnormal parts of the smear to guide manual reading.

Also for this system early non-randomized studies showed a much-improved screening performance compared to the manual reading of slides. A recent randomized study performed within the Finnish organized screening programme with high-quality manually-read cytology also found a (somewhat) improved detection of preinvasive lesions (RR=1.1). This moderate effect was seen only after 3 years of using automated reading on several hundred thousand slides.[256] Automatization of smear reading can decrease the costs of screening time considerably, yet the additional costs of processing and of equipment tend to neutralize this favourable effect.[257]

Even though the studies have so far not shown that either the LBC or automated screening perform substantially better in terms of improving the achieved standards of conventional manually-read cytology, they have some other advantages which make them an interesting option to consider for screening in the future. For example, the residual liquid of the LBC sample can be used for additional HPV testing, which would obviate scheduling and taking two separate tests in case the presence of an HPV infection would need to be determined. Also, it has been shown that both the LBC and automated screening can improve the output of cases per smear reader.[257, 258] These new methods, despite their less favourable cost-effectiveness ratio compared to conventional cytology,[142] could offer a solution to the Netherlands and other countries where a shortage of cytotechnicians is becoming a reality. Should the shortage continue to worsen, the drawbacks of these systems (the tendency to find more low-grade abnormal smears,[255, 259] higher costs[142]) should be properly addressed before their use becomes widespread.

HPV vaccination

The finding that cervical cancer cannot occur without an infection with one of the high-risk types of the Human Papillomavirus (HPV) started research into primary prevention of cervical cancer through HPV vaccination. Two vaccines (Gardasil and Cervarix) have been approved for market use in e.g. several EU countries and the USA. Both of these 1st generation prophylactic vaccines target the two HPV types (16 and 18) that are responsible for about 70% of the incident cases worldwide.[260, 261] Currently about five years of available follow-up (in the frequently screened trial participants) estimated the vaccine efficacy against persistent infection with HPV 16 or 18 at 76-100%, and against HPV 16 or 18-related CIN 2+ at 90-100%; while the potential for long-term cross-protection (i.e., against other oncogenic HPV types) may also play a role.[262-267] Because the highest grade of protection is achieved if women are vaccinated before their first infection with HPV 16 or 18,[262, 266, 268] the vaccine has thus far been approved for use only in girls and very young women.[269] The trials evaluating the efficacy of vaccination in older women are still in progress. Several issues that are crucial for an effective implementation of HPV vaccination programmes, e.g. the duration of the immune response, the rate of protection against cervical cancer, the long-term safety profile, etc. are still uncertain.[270-272]

HPV vaccination is going to play an increasingly important role in cervical cancer prevention. In the Netherlands, the Health Council in 2008 recommended to introduce this vaccine into the National Immunisation Programme for 12-year old girls despite its poorer cost-effectiveness compared to the current screening programme.[270] Even if vaccination will be introduced, it will not be able to completely replace screening. The implementation of the new combination of screening and vaccination will need to be properly planned, monitored and evaluated.[273]

Despite vaccination, screening will continue to play a role in cervical cancer prevention for two reasons. First, the vaccine should preferably be administered before the sexual debut, i.e. in early teenage years. The risk for cervical cancer for the majority of women already targeted by the screening programmes, and younger women who will be too old to be targeted by vaccination, will thus not be affected by the vaccine. Second, the vaccine does not protect against all high-risk HPV types. It is estimated that in Europe and Northern America the maximum achievable decrease in cervical cancer incidence through HPV vaccination is about 70%.[261] This maximum achievable effectiveness is dependent on high vaccination coverage.[274] There are other (theoretically possible) threats for the effectiveness of the vaccine. One is type replacement, a phenomenon whereby the niche of the vaccine-eradicated HPV-types could become re-populated with other oncogenic HPV types. Another is the development of oncogenic viral escape mutants, which would not be preventable by the vaccine. The likelihood of these phenomena to occur is considered to be low,[273] but will need to be monitored.

It is not yet determined what would be the optimal screening policy within the context of an effective HPV vaccination. Maintaining an equally intensive screening programme for the vaccinated women, however, would be very expensive and much less cost-effective than the current screening in non-vaccinated women. First, by the time the vaccinated women will reach screening age, their background risk for cervical cancer will be substantially lower than in unvaccinated cohorts. Second, the characteristics of cytology and HPV screening in vaccinated women may differ from screening in non-vaccinated women. HPV and cytology positivity in vaccinated women will decrease, but for HPV more so than for cytology.[275] As a consequence, in vaccinated women the specificity of HPV would improve compared to that observed in the non-vaccinated women. Moreover, in the context of a decreased PPV for high-grade lesions, the quality of smear-reading may deteriorate and consequently the sensitivity or specificity of cytology may decrease.[276] In case a considerable proportion of the population will be vaccinated, more research will need to be done into the optimal combination with screening.

For monitoring and evaluation, the data on all HPV tests as well as on HPV vaccinations should be registered in such a way that it could be readily linked to the cervical cytology and histology (PALGA) or other relevant registries.[277] In this respect it is a positive development that all HPV tests and their results will be routinely registered in PALGA. This is possible because (most of) the HPV tests are performed in pathology laboratories. For HPV vaccination, there is no registration set up yet, but should be established as soon as possible.

8.5. Conclusions and recommendations

1. The changes in the protocols and guidelines of the Dutch cervical cancer screening programme since 1996 helped to increase the coverage and to decrease the negative side effects of screening.
2. The shift of 80% of low-grade abnormal smears towards negative smears after 1996 did not measurably decrease the Pap smear sensitivity.
3. It is not consistent to stop screening women after they have had several consecutive negative smears by age 50, while not also relaxing the screening policy of similar younger women.
4. If the HPV test would replace the Pap smear in primary screening, the screening interval could be lengthened to more than the current 5 years.
5. Releasing HPV-negative women with a persistent low-grade abnormal smear to regular screening would reduce overtreatment without markedly decreasing the sensitivity of the screening programme.
6. Because cervical cancer prevention may undergo substantial changes in the near future, monitoring remains important.
7. Registration of HPV tests and HPV vaccination should be implemented either within PALGA, or readily linkable to PALGA at the level of an individual woman.
8. The expected increase in the screen-positivity rate in case of HPV testing should be critically assessed against the increase in the sensitivity.

8.6. Appendix: Trends in screening process indicators

Table 8-A1. 5-year coverage rates (in % of the population at risk)† with Pap smears in women aged 15-84 in the Netherlands in the period 1994-2006.[23, 93, 128]

	Age group																
Year	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84	20-29	30-64	65-84
1994	2	15	37	54	81	84	83	82	59	23	15	11	8	7	27	69	11
1995	1	14	36	53	82	83	83	81	59	25	15	11	8	7	26	69	11
1996	1	13	33	58	81	84	84	81	68	33	15	11	8	6	24	72	11
1997	1	11	29	64	81	85	85	82	76	45	15	11	8	6	21	76	11
1998	1	9	25	68	81	85	87	83	80	57	15	11	8	6	18	78	11
1999	1	8	21	71	82	85	86	84	81	70	15	10	8	6	15	80	10
2000	1	7	17	72	82	83	86	85	81	79	15	10	7	6	12	81	10
2001	1	6	15	71	80	82	84	85	79	77	15	9	7	6	11	80	10
2002	1	6	13	69	78	81	83	83	79	75	14	8	6	5	10	78	9
2003	1	5	12	68	77	81	82	81	80	74	14	8	6	5	9	78	9
2004	1	6	12	69	77	81	81	81	80	75	14	8	6	5	9	78	8
2005	1	6	12	69	78	81	81	81	79	76	15	8	6	5	9	78	9
2006	1	6	12	70	78	81	82	82	79	76	13	8	6	5	9	78	9

Shaded columns represent the post-1996 target age group. †Population at risk is the number of resident women on 1st January of the analysed year, excluding the estimated number of women who have undergone a hysterectomy. The estimated proportion of women with 0.5% most common surnames is excluded (based on the number of smears registered in PALGA in all vs. those women with 0.5% most common surnames; assuming that the commonness of surnames and screening behaviour are not correlated).

Table 8-A2. The participation rates in the Dutch cervical cancer screening programme, per age at invitation and year in the period 1996-2006.[23, 93, 128]

	Age at invitation							
Year	30	35	40	45	50	55	60	Total
1996	34%	38%	31%	16%	34%	28%	38%	31%
1997	40%	45%	40%	43%	39%	35%	43%	41%
1998	46%	51%	54%	52%	46%	50%	47%	50%
1999	51%	56%	61%	59%	55%	59%	53%	56%
2000	52%	60%	68%	65%	62%	66%	59%	61%
2001	51%	61%	67%	67%	64%	66%	64%	62%
2002	51%	62%	68%	69%	69%	67%	65%	64%
2003	52%	63%	69%	70%	71%	69%	66%	66%
2004	52%	61%	69%	70%	71%	70%	67%	66%
2005	52%	61%	68%	70%	71%	69%	68%	65%
2006	53%	61%	68%	70%	71%	70%	69%	66%

Excluding the estimated number of women who have undergone hysterectomy. Before 2000, 14%-31% of smears had no reason for smear-taking registered (Table 8-A5), thereby the participation rate is likely underestimated.

Table 8-A3. The type of advice given in the screening programme in the period 1990-2006, by screening year.[93]

Year	Total number of programme smears[†]	Follow-up advice					
		Next screening round (Pap 1)	Repeat smear (Ecc-)	Repeat smear (Pap 0)	Follow-up smear[‡]	Immediate referral[§]	Any follow-up advice
1990	131,372	76.6%	10.3%	1.6%	11.2%	0.3%	23.4%
1991	137,996	78.8%	9.1%	1.1%	10.7%	0.3%	21.2%
1992	152,463	78.9%	8.7%	1.2%	11.0%	0.3%	21.1%
1993	156,869	79.4%	8.0%	1.1%	11.1%	0.3%	20.6%
1994	162,537	81.1%	7.3%	1.3%	10.0%	0.3%	18.9%
1995	166,916	80.7%	7.8%	1.5%	9.8%	0.2%	19.3%
1996	218,807	84.8%	8.2%	1.0%	5.4%	0.6%	15.2%
1997	287,170	87.0%	7.8%	0.9%	3.7%	0.6%	13.0%
1998	351,675	88.7%	7.4%	0.9%	2.4%	0.6%	11.3%
1999	409,547	89.2%	7.2%	0.8%	2.1%	0.6%	10.8%
2000	442,767	89.0%	7.3%	1.0%	2.0%	0.6%	11.0%
2001	467,256	88.6%	7.7%	1.0%	2.1%	0.6%	11.4%
2002	481,483	96.4%	n.a.	1.0%	2.0%	0.6%	3.6%
2003	490,780	96.7%	n.a.	1.0%	1.8%	0.5%	3.3%
2004	497,023	96.6%	n.a.	1.1%	1.7%	0.6%	3.4%
2005	488,510	96.5%	n.a.	1.1%	1.9%	0.6%	3.5%
2006	507,536	96.0%	n.a.	1.4%	2.0%	0.6%	4.0%

Ecc-=negative smears without endocervical cells. n.a.=not applicable (Ecc- smears not followed-up between 2002 and 2006). [†]Primary smears labelled as due to the screening programme, registered from 1st January of the analysed year until 31st March of the following year, for the programme-eligible birth cohorts. [‡]1990-1995: Pap 2, Pap 3a1 and Pap 3a2; 1996-2006: Pap2 and Pap 3a1. [§]1990-1995: Pap 3b+; 1996-2006: Pap 3a2+.

Table 8-A4. Compliance with follow-up[†] in the screening programme, by type of recommendation and screening year.[93,‡]

Year	Type of follow-up smear advice		
	Immediate referral	Follow-up smear	Inadequate quality
1990	96%	83%	80%
1991	96%	85%	84%
1992	96%	83%	84%
1993	95%	76%	76%
1994	98%	78%	84%
1995	98%	78%	86%
1996	96%	70%	80%
1997	96%	69%	80%
1998	97%	85%	84%
1999	97%	90%	84%
2000	97%	90%	85%
2001	97%	90%	85%
2002	96%	91%	87%

Excluding women with 0.5% most common surnames. [†]At least cytology within 4 years of the primary smear. [‡]Abnormal smears taken between 1st January of the analysed year until 31st March of the following year, for the programme-eligible birth cohorts.

Table 8-A5. The total number of smears made (all ages),† and the reason for smear-taking (in % of the total) per calendar year in the period 1990-2006.[93]

		Reason for smear-taking (%)				
		Primary smears				Secondary smears
Year	Total number of smears	Screening programme	Opportunistic screening	Medical complaints	Unknown	
1990	964,585	21%	7%	4%	41%	27%
1991	964,641	23%	7%	4%	40%	26%
1992	956,045	24%	8%	4%	39%	26%
1993	937,055	23%	7%	5%	39%	26%
1994	990,261	23%	6%	7%	39%	25%
1995	971,130	24%	6%	7%	37%	26%
1996	1,073,447	35%	4%	6%	31%	24%
1997	1,086,863	39%	3%	6%	28%	24%
1998	993,357	47%	3%	7%	22%	21%
1999	897,815	54%	3%	10%	14%	19%
2000	830,381	60%	2%	11%	9%	17%
2001	843,022	62%	2%	12%	9%	15%
2002	810,015	66%	3%	14%	9%	9%
2003	797,526	67%	2%	13%	9%	9%
2004	824,046	67%	2%	13%	9%	8%
2005	789,717	68%	2%	13%	8%	8%
2006	807,082	68%	2%	14%	8%	9%

†Adjusted for population growth (20-84 years) relative to 2006.[23]

Table 8-A6. The total number of smears made in women aged 20-29, and in % of the total number of smears, per calendar year in the period 1990-2006.[93]

Year	Smears at age 20-29	Smears at age 20-29, adj.†	Proportion of all smears
1990	150,345	116,658	17%
1991	144,390	112,216	16%
1992	139,161	108,823	16%
1993	129,662	102,940	15%
1994	130,459	105,767	14%
1995	118,856	99,044	13%
1996	108,699	93,091	11%
1997	105,971	93,051	10%
1998	91,431	82,341	10%
1999	82,588	76,611	10%
2000	56,755	54,004	7%
2001	52,269	50,691	6%
2002	46,672	46,017	6%
2003	44,373	44,191	6%
2004	45,853	45,920	6%
2005	45,250	45,327	6%
2006	48,249	48,249	6%

†Adjusted for the growth of the population aged 20-29 (2006=index year).[23]

CHAPTER 9. Summary - Samenvatting

Summary

From 1996 onwards, new cervical cancer screening protocols and guidelines were implemented because the existing screening practice (the loosely organized programmes and spontaneous screening) was far from optimal. The organized programmes were changed into one national programme in which women are invited at 5-yearly intervals between ages 30 and 60. Smears are taken at the general practitioner's (GP) practice, and are free of charge. Screening outside of the programme has been discouraged. National guidelines were published for GPs, pathologists and gynaecologists. The programme is financed by earmarked national funds, and implemented by 12 regional screening organisations. The first part of this thesis presents several aspects of the functioning and effectiveness of the programme, evaluated recently at the national level.

In the late 1990's, it was confirmed that an infection with oncogenic Human Papillomavirus (HPV) is a necessary cause of cervical cancer. This spawned interest into both new tools for early detection of cervical cancer (HPV test), as well as into primary prevention through HPV vaccination. The second part of this thesis presents recent work exploring the possibilities of using the HPV test in in primary screening, and in triage of low-grade abnormal smears.

The screening process

In **Chapter 2**, we analysed the changes in the screening programme by 2003 in terms of the coverage, resource use and side effects. These indicators give an early signal whether the programme is achieving its goal of mortality reduction, and help assess the screening-induced burden on screened individuals. We showed that there have been considerable improvements in the screening programme on both counts. First, the 5-year coverage rate is now close to 80%. Since 1994, it increased especially in the added target age groups (30-34 and 54-64 years). Second, the new screening protocols and guidelines brought about a considerable decrease in the resource use (a 20% overall decrease in smears made per year, especially among women below 30 years), and a decrease in screening-induced side effects (an approximately 80% decrease in the programme smear positivity rate, and much quicker decision-making for women with positive screening smears).

The broadened definition of a negative smear

A change in the classification of inflammation from abnormal to normal caused an approximately 70% overall decrease in the number of women with a follow-up advice due to a positive smear. In **Chapter 3**, we showed that this was not associated with a measurable decrease in smear sensitivity: within 6 years of a negative smear, the incidence of cervical cancer after a negative smear was similar in the periods before and after 1996. This suggests that inflammation (without concurrent signs of dysplasia) is not a risk factor for cervical cancer.

Screening after several consecutive negative smears by age 50

Fewer CIN lesions are detected in women who had several negative smears by age 50 compared to similar younger women. This has been an argument calling to cease screening in these women. Using CIN data in age comparisons, however, is problematic because the progression rate of CIN lesions to cancer increases with age. In **Chapter 4**, we showed that the incidence of cancer after the third consecutive negative smear around age 50 is the same as after the third consecutive negative smear in younger women. This finding showed that it would not be consistent to stop screening in women with several consecutive negative smears at age 50, while not relaxing the screening policy for similar younger women.

HPV testing in primary screening

In order to assess the differences in their negative predictive value, we compared the incidence rates of CIN 3+ after a negative HPV test and a negative Pap smear. In **Chapter 5**, we analysed a large data set composed of seven recently finished European trials, in which women were screened with the Pap smear and the HPV test, and thereafter followed for several years. We found that within 6 years of a negative HPV test fewer women develop a CIN 3+ lesion than within 3 years of a negative Pap smear. We conclude that in case the HPV test would substitute the Pap smear as the primary test, the screening interval could be safely prolonged to more than the current 5 years. However, the advantages of the HPV test over the Pap smear in terms of higher sensitivity will have to be balanced with the disadvantages in terms of lower specificity and possibly higher costs.

HPV testing in triaging of women with persistent low-grade abnormal smears

The standard follow-up recommendation for women with a persistent low-grade abnormal smear was a referral to a gynaecologist. Because the majority (80%) of these women do not have a high-grade CIN (CIN 2+) lesion, a significant burden was induced on women and the health care. In **Chapter 6** and **Chapter 7**, we explored whether instead of the standard recommendation a high-risk selection could be made based on the HPV test. In the trial, almost all high-grade CIN lesions were found in HPV-positive women, whereas the negative predictive value of a negative HPV test was close to 100%. Compared to the standard recommendation, triage by HPV testing resulted in a decrease in the total costs, and an alleviation of the screening burden. In view of simplicity for both physicians and women, it is preferable to combine the HPV test with the first follow-up smear.

Conclusion

The implementation of the new guidelines and protocols has improved the Dutch cervical cancer screening programme considerably. The coverage rate increased in the previously under-screened age groups, whereas the abnormality rate decreased. In the future, the programme may change due to newer early detection techniques and tools for cancer prevention. HPV

testing seems promising in triage of low-grade abnormal smears. For primary screening, the balance between a higher sensitivity but lower specificity and higher costs of the HPV test compared to the Pap smear will have to be assessed in a cost-effectiveness analysis.

Samenvatting

In 1996 zijn nieuwe protocollen en richtlijnen geïmplementeerd voor de screening op baarmoederhalskanker. De bestaande screeningpraktijk was destijds verre van optimal. De georganiseerde screening werd nationaal gestandaardiseerd tot een bevolkingsonderzoek waarin vrouwen in de leeftijdsgroep 30-60 jaar iedere 5 jaar werden uitgenodigd om bij de huisarts een (gratis) uitstrijkje te laten maken. Spontane screening werd ontmoedigd. Voor huisartsen, pathologen en gynaecologen werden nieuwe nationale screeningsrichtlijnen opgezet. Het bevolkingsonderzoek werd gefinancierd uit speciale fondsen door de overheid en geïmplementeerd door 12 regionale screeningsorganisaties. In het eerste gedeelte van dit proefschrift worden verschillende aspecten gepresenteerd met betrekking tot het functioneren en de effectiviteit van het nieuwe bevolkingsonderzoek dat recent op nationaal niveau is geëvalueerd.

Eind jaren 90 werd bekend dat besmetting met het Humaan Papillomavirus (HPV) een voorwaarde vormt voor het ontstaan van baarmoederhalskanker. Dit bracht interesse naar voren voor de ontwikkeling van zowel nieuwe methoden van vroege opsporing van baarmoederhalskanker als voor primaire preventie via vaccinatie tegen HPV. In het tweede gedeelte van dit proefschrift wordt recent werk gepresenteerd waarin de mogelijkheden worden onderzocht van het gebruik van HPV testen bij primaire screening en bij triage van licht afwijkende uitstrijkjes.

Het screeningsproces

In **Hoofdstuk 2** wordt het bevolkingsonderzoek van 2003 geëvalueerd op de indicatoren: bereik, inzet van middelen (aantal benodigde uitstrijkjes) en neveneffecten. Deze indicatoren geven een vroeg signaal af of het screeningsprogramma sterftereductie bereikt en hoe groot de belasting voor de doelgroep is. Op beide punten zijn forse verbeteringen zichtbaar ten opzichte van de situatie voor 1996. Ten eerste is het 5-jaars bereik nagenoeg 80% geworden. Sinds 1994 stijgt de dekking met name in de toegevoegde doelgroep (30-34 jaar en 54-64 jaar). In de tweede plaats is dankzij de nieuwe richtlijnen het middelengebruik van screening naar baarmoederhalskanker gedaald (er worden jaarlijks ongeveer 20% minder preventieve uitstrijkjes gemaakt) en de ongewenste neveneffecten van screening naar baarmoederhalskanker zijn verminderd (80% minder fout positieve uitslagen en een sneller vervolgtraject voor vrouwen met een afwijkend uitstrijkje).

De bredere definitie van een negatief uitstrijkje

Een verandering in de classificatie van ontstekingsverschijnselen van afwijkend naar normaal leidde tot 70% afname van het aantal vrouwen dat bij het bevolkingsonderzoek een advies voor een herhalingsuitstrijkje kreeg. In **Hoofdstuk 3** laten we zien dat naar aanleiding hiervan de sensitiviteit van het uitstrijkje niet verminderde: de incidentie van baarmoederhalskanker binnen 6 jaar na een negatief uitstrijkje was gelijk in de periode voor en na 1996. Dit geeft aan dat ontstekingsverschijnselen (zonder aanwezige kenmerken van dysplasie) geen risicofactor zijn voor baarmoederhalskanker.

Screening na een aantal opeenvolgende negatieve uitstrijkjes op 50-jarige leeftijd

In vergelijking met vergelijkbaar gescreende jongere vrouwen worden er minder CIN laesies gevonden bij vrouwen die rond hun 50ste een screeningsgeschiedenis van meerdere achtereenvolgende negatieve uitstrijkjes hebben gehad. Dit is een argument om screening bij deze vrouwen te stoppen. Het gebruik van data over CIN bij vergelijkingen tussen leeftijdsgroepen is problematisch omdat de kans dat CIN progressief is toeneemt met het stijgen van de leeftijd. In **Hoofdstuk 4** laten we zien dat de incidentie van baarmoederhalskanker na het derde achtereenvolgende negatieve uitstrijkje rond de leeftijd van 50 identiek is aan de kans op kanker na drie negatieve uitstrijkjes bij jongere vrouwen. Deze bevinding geeft aan dat het niet consequent is om bij vrouwen rond de 50 te stoppen met screening na drie negatieve uitstrijkjes en niet bij vergelijkbare jongere vrouwen het screeningsbeleid te versoepelen.

HPV testen in primaire screening

Om het verschil in de negatief voorspellende waarde te bepalen hebben we de incidentie van CIN3+ na een negatieve HPV test en na een negatief uitstrijkje met elkaar vergeleken. In **Hoofdstuk 5** hebben we een grote dataset geanalyseerd bestaande uit 7 recent afgeronde Europese trials, waarin vrouwen werden gescreend met zowel een uitstrijkje als een HPV test en vervolgens verschillende jaren werden gevolgd. We vonden dat binnen 6 jaar na een negatieve HPV test minder vrouwen een CIN 3+ laesie ontwikkelden dan binnen 3 jaar na een negatief uitstrijkje. We concluderen dat in het geval dat de HPV test in plaats van het uitstrijkje als primaire test gebruikt zou worden, het screeningsinterval langer zou kunnen worden dan de huidige 5 jaar. Desalniettemin moeten de voordelen van de HPV test in vergelijking met het uitstrijkje op het gebied van hogere sensitiviteit zorgvuldig worden afgewogen tegen de nadelen waaronder een lagere specificiteit en hogere kosten.

HPV testen bij triage van vrouwen met persistente licht afwijkende uitstrijkjes

Het gebruikelijke follow-up advies voor vrouwen met persistente licht afwijkende uitstrijkjes was een verwijzing naar de gynaecoloog. Omdat de meerderheid (80%) van deze vrouwen geen CIN2+ heeft is dit een behoorlijke belasting voor de betreffende vrouwen en voor de gezondheidszorg. In **Hoofdstuk 6** en **Hoofdstuk 7** hebben we onderzocht of het gebruikelijke follow-up advies kan worden vervangen door een risicoselectie te maken op basis van de HPV test. In de trial werden nagenoeg alle vrouwen met CIN2+ ook positief gediagnosticeerd op basis van de HPV test terwijl de negatief voorspellende waarde dicht bij de 100% lag. Vergeleken met de standaard richtlijn resulteert triage met HPV tests in een afname van de totale kosten en een vermindering van de belasting voor de betreffende vrouwen. Vanuit het oogpunt van gemak voor zowel vrouwen als artsen is een combinatie van een uitstrijkje en een HPV test bij het eerste herhalingsuitstrijkje aan te bevelen.

Conclusie

De implementatie van de nieuwe richtlijnen en protocollen hebben de praktijk van screening op baarmoederhalskanker in Nederland sterk verbeterd. Het bereik is toegenomen binnen de voorheen ongescreende leeftijdsgroepen, terwijl het percentage afwijkende uitstrijkjes sterk daalde. In de toekomst kan het bevolkingsonderzoek veranderen door het gebruik van nieuwe preventie- en opsporingstechnieken. HPV testen lijken veelbelovend bij de triage van licht afwijkende uitslagen van het uitstrijkje. Voor primaire screening moet de balans tussen een hogere sensitiviteit maar een lagere specificiteit en hogere kosten van de HPV test in vergelijking met het uitstrijkje onderzocht worden in een kosten-effectiviteitsstudie.

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(Kajetan Kovič)

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And many grown-up children
And so many small, minuscule things
And so many helping hands.

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Say cheerio to books now
The only books I'll read are faces.
(Belle and Sebastian)