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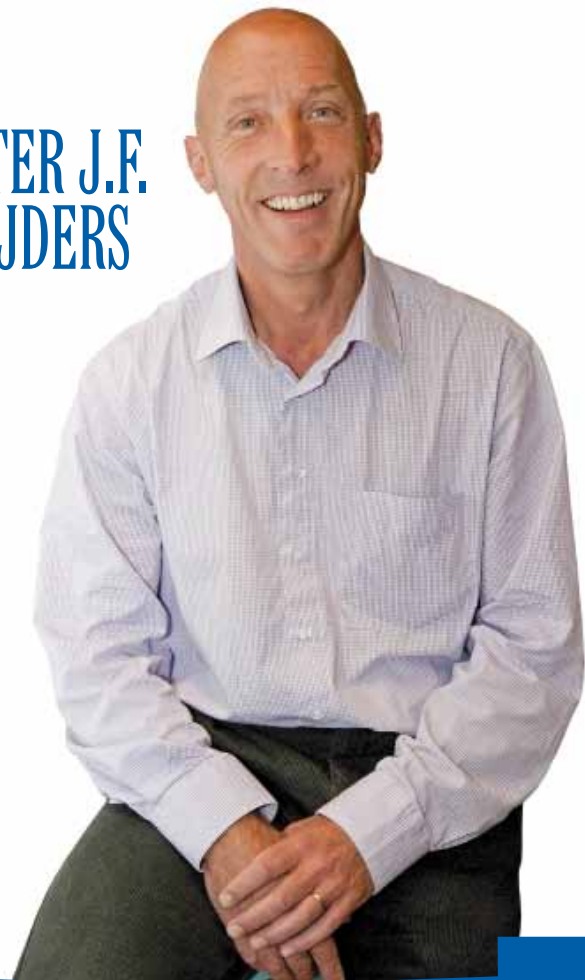
THE DUTCH HEALTH COUNCIL HAS ADVISED THE MINISTRY OF HEALTH TO INTRODUCE PRIMARY HPV TESTING IN ITS POPULATION-BASED CERVICAL CANCER SCREENING PROGRAM. THE MINISTRY IS CONSIDERING A FORMAL IMPLEMENTATION TEST

This advice is based on the large body of scientific evidence that has accumulated over more than 25 years.

Functional studies in the 1980s and early 1990s established the **transforming properties of high-risk HPVs (hr-HPVs)** by means of viral E6 and E7 oncogenes, and provided evidence that maintenance of the transformed phenotype depends on continuous viral oncogene activity. This milestone subsequently led to numerous epidemiological studies on the relationship between HPV infections and cervical (pre) cancer and the **development of consensus Polymerase Chain Reaction (PCR) methods** (such as General Primer (GP5/6-PCR, which was originally developed in our laboratory¹) that enabled detection of a broad spectrum of genital HPV types with high sensitivity. The application of these methods to case series collected by Nubia Muñoz and co-workers at the International Agency Research on Cancer (IARC) ultimately led to the landmark paper of Jan Walboomers and co-workers which showed that **hr-HPV is present in 99.7% of cervical carcinomas worldwide** and the subsequent conclusion that infection with hr-HPV is a necessary cause of cervical cancer.² **The group of hr-HPV types was subsequently defined** on the basis of epidemiological criteria using pooled data from worldwide IARC case-control studies.³ The prevalence of this sexually transmittable virus in the Netherlands and many other Western countries peaks in

(continues on page 3)

PETER J.F.
SNIJDERS



MILESTONES IN A DECISION PROCESS

CYTOLOGY REMAINS THE OPTION OF CHOICE FOR TRIAGE AND MANAGEMENT OF HPV-POSITIVE WOMEN

THE LONG ROAD FROM PAP SMEARS TO HPV TESTS AND WHAT COMES NEXT

Preventive medicine has traditionally been slow to introduce its postulates when it comes to modifying the practices and habits of the general population. Indeed, several decades elapsed between the original work of Drs. Babes, Papanicolau and Traut in the early 1940s and the organization of centralized population-based screening programs with good coverage. In most developed countries, the practice of screening is still a mixture of publically supported programs and insurance-funded protocols in private practice, which results in irregular coverage of the at-risk population, limited efficiency and lack of systematic quality controls. Furthermore, the inability of cytology to consistently distinguish the earlier forms of Cervical Intraepithelial Neoplasia (CIN) and the different classifications used result in a significant number of screening failures. Diagnostic uncertainties are prone to generate over-diagnosis and treatment of what we now recognize as common acute HPV infections that are largely destined to regress. Despite such limitations, reductions of up to 70% in cervical cancer incidence and mortality have been achieved in some developed countries using the Pap Smear.

HPV tests suitable for clinical use first became available in the 1990s, and a number of subsequent studies and clinical trials proved beyond reasonable doubt that a single HPV test provided significantly higher sensitivity and positive predictive values, with a moderate loss in specificity for CIN2+, than the conventional Pap smear. However, it has taken at least another decade for the first Health Council of a European country to recommend to its Ministry of Health that the Pap smear be replaced by HPV testing as the primary screening option. This recommendation also provides evidence that, compared to several alternatives, cytology is currently the triage test of choice when deciding on the management of women found to be high-risk (hr)-HPV positive. Controlled trials and model analyses were instrumental in supporting this landmark evidence-based recommendation. Better screening tools will allow late initiation of screening (particularly in vaccinated cohorts) and less frequent screening rounds without compromising security. Furthermore, there will be a global economy in the system in terms of medical and women's time as well as a reduction in the total number of medical events required for a better protection against cervical cancer.

How can the time gained be best used?

In developed countries, publically supported regular visits to gynaecologists are a unique achievement of preventive medicine. The precious time gained by releasing the pressure imposed by the repetitive Pap-based screening protocols can now be devoted to better address the cancer priorities in each country. Indeed, breast, colorectal and lung cancers are nowadays the major cancer killers among women in most countries. Ensuring coverage and quality for breast and colorectal cancer screening, as well as smoking cessation protocols, could represent a new breakthrough in the preventive capacity of our health services. Research for better prevention of these cancers and, more generally, into other health priorities for each age group can now find a golden opportunity, driven once more by the professionals who first introduced generalized cervical cancer screening.

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MILESTONES IN A DECISION PROCESS

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younger women, but drops considerably in women of screening age (30 years and older in the Netherlands).⁴

Together with natural history data showing that **persistence of an hr-HPV infection is essential for the development, maintenance and progression of high-grade cervical intraepithelial neoplasia (CIN)**,^{5,6} all ingredients were present to consider HPV testing as a conjunctive or alternative cervical screening tool.

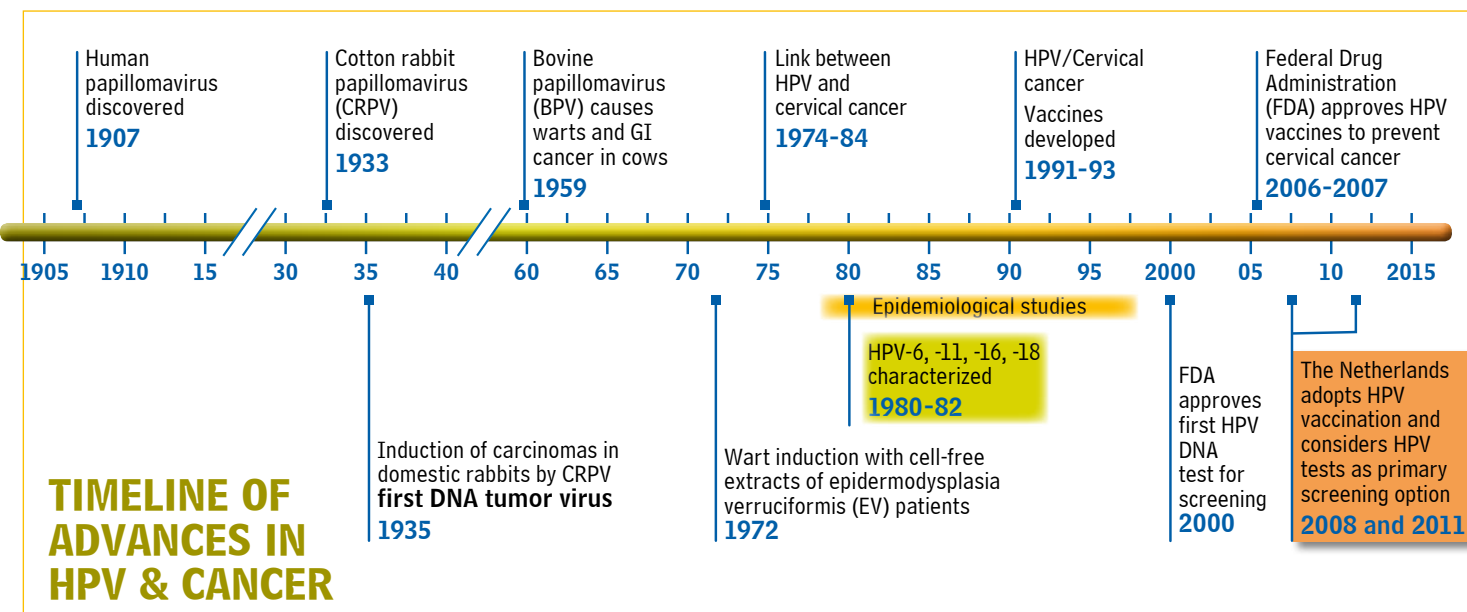
The multitude of cross-sectional studies undertaken since then have shown that **HPV testing is about 45% more sensitive than cytology in detecting high-grade CIN lesions**. However, definitive proof of improved protection against high-grade CIN and cervical cancer came from longitudinal, randomized-controlled trials (such as the Population-based screening study Amsterdam-POBASCAM, the Swedish nationwide population-based cohort

when considering fewer screening rounds to save costs.¹⁰

An important observation that has arisen in the last few years is that various HPV detection methods differ in their clinical performance, with the specificity for CIN2+/CIN3+ often varying substantially¹¹ This reflects the fact that detection of very low viral copy numbers in cervical scrapings does not result in better detection of high-grade cervical disease but rather the presence of clinically irrelevant infections. Methods should therefore not be too sensitive for detecting HPV in order to reduce the number of false-positives (high-risk HPV-positives without high-grade disease) and redundant and unnecessary follow-up procedures. To avoid the use of poorly performing HPV tests in primary cervical screening, the criteria for HPV test requirements have been formulated in guidelines and translated into a clinical

validation procedure by an international consortium.¹² Finally, an important spin-off of HPV research is that an increased screening attendance can also be reached when HPV testing is considered in the context of self-sampling. **Recent large studies on screening non-attendees have demonstrated that offering self-sampling for HPV testing is an effective approach that attracts up to 30% of non-attendees into the screening program.**¹³⁻¹⁵ In combination with newly developed molecular triage methods¹⁶ that are applicable to self-sampled specimens, this opens up the possibility of an easily accessible screening format. Researchers from the Netherlands were involved in many of the studies cited above and data were obtained from Dutch women. It is therefore not surprising that the Netherlands will probably be the first country to formally adopt HPV testing as the primary tool for organised cervical screening.

TIMELINE OF ADVANCES IN HPV & CANCER



study-SWEDESCREEN and the new technologies for cervical cancer-NTCC study) in which women were followed over two screening rounds.⁷⁻⁹ **These trials revealed an average reduction of CIN3+ lesions of about 50% in the HPV arm in the second screening round**, and one trial (NTCC) was sufficiently powerful to also show a significantly better prevention of cervical cancer in the HPV arm.⁹ Furthermore, the studies that used HPV and cytology co-testing in the experimental arm also concluded that **HPV testing alone is just as sensitive for high-grade CIN and cervical cancer as the combined HPV and cytology test**. This finding argued strongly for the use of HPV testing as a stand-alone test for primary cervical screening. More importantly, modeling studies revealed that, in the Netherlands, primary HPV testing with cytology triage for HPV-positive women could be effective and cost-neutral when compared with cytology-based screening

validation procedure by an international consortium.¹² Finally, an important spin-off of HPV research is that an increased screening attendance can also be reached when HPV testing is considered in the context of self-sampling. **Recent large studies on screening non-attendees have demonstrated that offering self-sampling for HPV testing is an effective approach that attracts up to 30% of non-attendees into the screening program.**¹³⁻¹⁵ In combination with newly developed molecular triage methods¹⁶ that are applicable to self-sampled specimens, this opens up the possibility of an easily accessible screening format. Researchers from the Netherlands were involved in many of the studies cited above and data were obtained from Dutch women. It is therefore not surprising that the Netherlands will probably be the first country to formally adopt HPV testing as the primary tool for organised cervical screening.

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CHANGING THE PRIMARY SCREENING TOOL OF THE PROGRAM IN THE NETHERLANDS. WHY AND HOW?

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On March 20, 2007, the Dutch Ministry of Health asked the Health Council whether:

1. prophylactic vaccination should be introduced into the Dutch population, and if so, at what age, and
2. the present screening program should be improved.

On July 10, 2007, the Health Council established the cervical cancer prevention committee to prepare the corresponding advice. On March 31, 2008, the Health Council released the first part of its advice in favour of vaccinating young, HPV-naïve women with the HPV-16/-18 L1 virus-like particle (VLP) vaccine. This vaccination was recommended to be given at the age of 12, with catch-up to 16 years. The committee published the second part of its advice on May 24, 2011.

An organized screening program has been in place in the Netherlands since the 1970s. The last revision to this program was introduced in 1996 and contained the following modifications:

1. The age range was extended (starting at age 30 years from 35) and the last screening episode was put back to 60 years of age (from 55);
2. The screening interval was extended from three to five years; and
3. Abnormal smears were classified according to the KOPAC-B (CISOE A) classification (C: composition; I: inflammation; S: squamous; O: others, including endometrium; E: endocervix; A: adequacy).¹ Each of these items is scored on a scale from 0-9 and a computerized algorithm is used to generate the new Pap classification and provide the referral advice. This classification can easily be translated into the Bethesda classification and has a high reproducibility.¹ Introduction of this classification has resulted in a decrease in the percentage of smears with minor cellular abnormalities (borderline and mild dyskaryosis (BMD), equivalent to Abnormal squamous cells of undetermined significance (ASCUS)/low squamous intraepithelial lesions (LSIL) from 11% to 2.3% with no decrease in the detection of cervical intraepithelial neoplasia of grade 3 (CIN3) lesions, and a decrease in the incidence and mortality of cervical cancer. The program has 67% attendance and, together with opportunistic and diagnostic smears, a coverage of 75%; its annual cost is around 35 M€. Some 450,000-500,000 smears are performed per year within the program and 100,000-150,000 smears outside the program.

Why should we change this program?

The Health Council committee evaluated the activity, effectiveness and efficiency of the present cytology-based program and decided where it could be improved. The following points were noted:

- a. In the cytology-based screening program, women with BMD can have follow-up tests at 6 and 18 months before a definite result, in other words referral for colposcopy or return to the screening program, is obtained. Women are therefore lost to follow-up and suffer from stress whilst waiting for the test result.
- b. The sensitivity of cytology for CIN2+ is too low, thereby resulting in at least 10% false-negative smears; false-positive smears also occur.
- c. The incidence of squamous cell carcinoma has not decreased since 2004,² and adenocarcinomas are missed. Indeed, the incidence of adenocarcinoma has never decreased since the screening program was first introduced.³
- d. The attendance of the screening program (67%) can be improved.

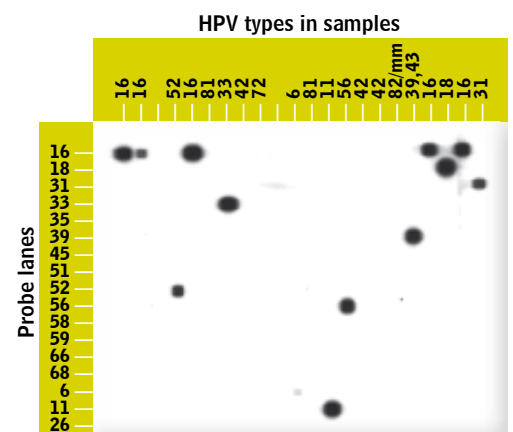


Figure:

Examples of HPV test readouts for HPV +/- result (panel a) and for a type-specific result (panel b): Molecular assays are less ambiguous in terms of interpretation than the conventional Pap smear.

a: Enzyme immunoassay of General Primer (GP)5+/6+-Polymerase Chain Reaction (PCR) products using a high-risk probe pool

b: Reverse line blot genotyping of GP5+/6+-Polymerase chain Reaction (PCR) products



In light of these observations, the committee noted that:

- a. HPV tests have been developed for cervical screening since it first became clear that high-risk (hr)-HPV infection is the necessary cause of cervical cancer.
- b. The results of several large trials⁴⁻⁶ have shown that hr-HPV testing is about 50% more sensitive for the detection of high-grade CIN and that the negative predictive value of an hr-HPV test for CIN2+ is about 6% higher than that of a negative cytology test. Even more importantly, in the second round (three to five years later) those women who tested negative for hr-HPV in the first round of these trials had a 50% lower incidence of CIN3 than cytology-negative women,⁴⁻⁶ and fewer carcinomas were found in the hr-HPV arms, with this difference being statistically significant in the New technologies for cervical cancer (NTCC) trial in Italy.⁶
- c. The reproducibility of hr-HPV testing is much higher than that of cytology, and hr-HPV test guidelines have been published in which the conditions that have to be fulfilled in order for hr-HPV tests to be used in routine screening have been described. Those tests that fulfill these conditions are defined as clinically validated.⁷
- d. Cost-effectiveness and cost-efficiency studies have revealed that screening intervals can be extended without increasing the CIN2+ interval risk when an hr-HPV test is used as the primary screening test.⁸ If the present number of screening rounds can be reduced to five, hr-HPV-based screening will be cost-neutral compared to the present cytology-based screening program. In contrast, if six rounds are used the program will be around 5 M€/year more expensive.
- e. The higher sensitivity and somewhat lower specificity for CIN2+ will result in a threefold higher referral rate to colposcopy compared to the present cytology-based program. Triage of hr-HPV-positive women by cytology at baseline and after six months is therefore recommended, with referral to colposcopy if cytology is abnormal (threshold \geq BMD). Cytology is easy to implement as a triage test. The five-year CIN2+ risk for hr-HPV-positive women with two negative cytology tests is 0.8%, which is lower than the accepted risk of <2% for population-based screening.⁹
- f. Screening by primary hr-HPV testing will start at the age of 30 years. Although the prevalence of hr-HPV at this age is 8%, the harvest of CIN2+ in the 30-35 years age group is quite high, and data from the Population-Based Screening Study Amsterdam (POBASCAM) study show that the large majority of these lesions do not regress.

In light of these arguments and facts, the Health Council decided to advise the Minister of Health to implement hr-HPV testing as the primary screening test in cervical cancer screening for women aged 30-60 years, with cytology triage for those testing positive. Furthermore, although the Health Council noted that several screening schemes can be used, it suggested that screening be performed at 30, 35, 40, 50 and 60 years of age.

Women who are screened at 40, 50 and 60 years and are hr-HPV-positive at baseline and cytology-negative at both baseline and 6 months are retested at 45, 55, and 65 years by hr-HPV testing and cytology triage at baseline and after 6 months. In practice, this means that women are under the protection of the screening program up until 65 years of age.

To increase the attendance of the screening program, and in light of the results of the Protection by offering HPV testing on cervicovaginal specimen Trial (PROHTECT) studies,^{10,11} the committee has advised to send a device for self-sampling cervico-vaginal material for hr-HPV testing to those women who do not respond to a screening invitation or a repeat invitation after 3-6 weeks.

These studies have found that 27-30% of non-responding women in regular screening will participate when a self-sampler is sent to them.

Self-sampling thus increases the overall sensitivity of the screening program. The Health Council has not yet adopted the use of self-sampling devices as a primary possibility in regular screening, and recommends further investigation in clinical trials as an alternative to a physician-taken cervical scrape in regular screening.

With this screening program, the Netherlands will be the first country with an hr-HPV-based screening program and triage with cytology that involves only five lifetime screening rounds.

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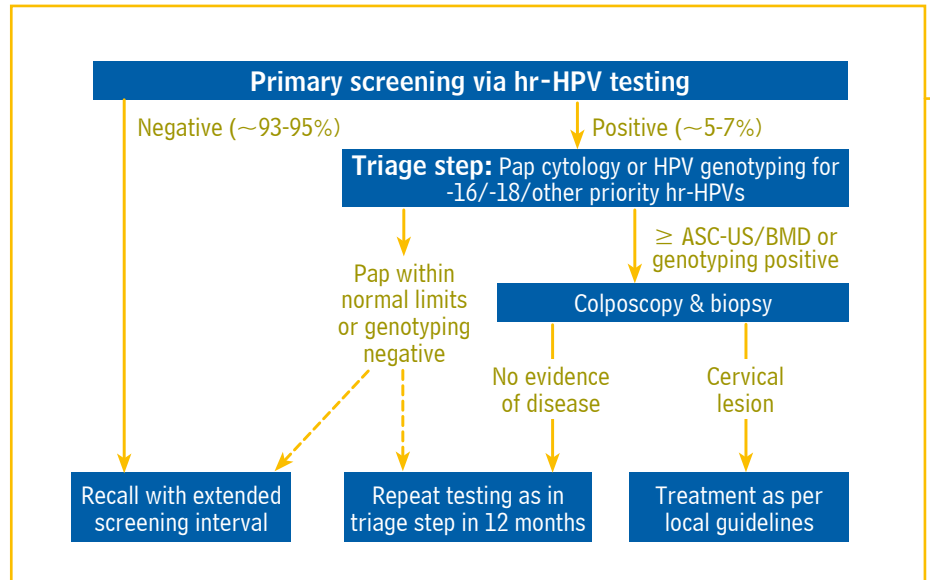
CERVICAL CANCER SCREENING IN WOMEN WHO WERE VACCINATED AGAINST HPV INFECTION

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The Netherlands is now entering the annals of public health history as a pioneer in the implementation of molecular HPV testing as a primary cervical cancer screening tool. The Dutch have already championed the cause of cervical cancer prevention by incorporating HPV vaccination into their national immunization programme, and now, as a result of strong science, effective advocacy, and political courage, they will undoubtedly reap the benefits of acting comprehensively on these two disease-prevention fronts. HPV Today's readers are well aware that the two HPV vaccines that have passed the regulatory hurdles, namely Merck's Gardasil® and GSK's Cervarix®, protect against acquisition of infections with oncogenic HPV types -16 and -18 and their lesions, which are responsible for about 70% of all cervical cancers. **However, although second-generation vaccines will have enhanced coverage, screening will have to continue as an essential public health strategy for the next several decades.** The question that now remains is: how can screening and vaccination work together to maximize health benefits whilst minimizing costs to the Dutch healthcare system?

The traditional paradigm of Pap cytology as a primary screening tool will not be a suitable complementary preventive strategy in the era of HPV vaccination. Indeed, once the birth cohorts of women who are currently being vaccinated reach screening age, the prevalence of Pap smear-detectable abnormalities will decrease substantially, thereby ultimately affecting the positive predictive value of cytology and decreasing its cost-effectiveness. Although this problem would also affect any screening test, cytology is likely to be affected more acutely than other technologies because of the subjective nature of interpreting smears. The molecular testing of cervical exfoliated cells for nucleic acids from oncogenic, high-risk (HR) HPVs, as pioneered by the Dutch in the early 1990s¹,



is a much more sensitive screening tool than cytology for detecting high-grade cervical lesions and cervical cancer in women 30 years or older. hr-HPV testing is also more reproducible and adaptable to automation than cytology, thus making it a more robust technology for wide-scale implementation. **However, the fact that hr-HPV testing also detects transient infections would make screening based exclusively on single-step presumptive testing too onerous in terms of colposcopy referrals.**² A triage step for women who are hr-HPV positive is thus necessary. Using data from the Dutch VUSA-Screen study, Rijkaart et al.³ examined a variety of options for triaging high-risk (hr)-HPV-positive women and concluded that cytology triage can detect those who should undergo colposcopic examination and biopsy or be surveyed more closely, which largely assuages the concerns related to excessive referrals and false-positives. The improved sensitivity for detecting existing lesions, and the more "upstream" focus on cervical carcinogenesis, would permit this strategy to be implemented via longer screening intervals than are currently possible with cytology alone, thus meaning that it would be cost-saving, especially after hr-HPV testing is deployed as a screening

tool. Other options for triaging hr-HPV-positive women include risk stratification with HPV genotyping for HPV-16 and -18 or for HPV-16, -18, -31, -33, and -45.³ It is in the post-vaccination era, when the cohorts of women vaccinated in their teens enter screening age, that this approach may prove most valuable by serving as a surveillance system that can fulfill two roles simultaneously: monitoring the duration of vaccine protection (with HPV genotyping for those who are positive) and screening for cervical cancer. The generic algorithm shown in Figure 1 illustrates how a combined hr-HPV testing strategy, followed by triage and follow-up testing steps based on cytology or genotyping for the most oncogenic HPV types, could serve this dual role. This approach may prove to be cost-effective in high-resource countries by permitting the integration of current immunization practices and cervical cancer control programmes, thus favouring the sharing of resources and surveillance infrastructure. The establishment of vaccination registries that can be linked to administrative databases of cervical screening use and tumor registries is an essential requirement for this process to become an effective monitoring system post-HPV vaccination.



NOVEL OPTIONS FOR TRIAGING HPV-POSITIVE WOMEN WITH MOLECULAR MARKERS

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Figure 1.

A generic algorithm describing a suitable strategy for cervical cancer screening that could serve an additional role as a surveillance system post-HPV vaccination in high-resource settings. An organized programme is assumed. Primary screening via hr-HPV testing with a validated assay begins at age 25 years (or age 30), i.e., approximately 10-20 years after school-based HPV vaccination. Women with a negative result would be re-screened via an extended interval (relative to that of Pap cytology-based screening). The definition of extended interval can be tailored to suit societal preferences. A triage step for women who test positive includes either Pap cytology (conventional or liquid-based) or genotyping for HPV-16 and -18 or for HPV-16/-18/-31/-33/-45 (as studied by Rijkaart et al., 2011). A positive result in the triage step would trigger a colposcopy referral, and any disease detected at that point would be treated according to local practices. For safety, women deemed lesion-free on colposcopy would be subjected to the same testing approach used at the triage step after 12 months. If they are negative on follow-up, they would be managed via the extended interval (left-hand side); if positive, they would undergo colposcopy again. Depending on locally built consensus, women with negative triage results (broken line) could return to the extended screening interval or undergo testing as in the triage step 12 months later. If negative at that point, they would be directed to the extended interval. Abbreviations: ASC-US, atypical squamous cells of undetermined significance; BMD, borderline or mild dyskaryosis; hr-HPV, high-risk HPV. The repeat testing at 12 months could be reduced to six months to comply with practice preferences for risk avoidance. Conversely, a higher cytological grade threshold could be used in the triage or follow-up steps, i.e., low-grade squamous intraepithelial lesion (LSIL). The percentages on each side of the split following the primary screen with hr-HPV indicate the proportions of screening participants aged 30 and older with the specified hr-HPV test result, based on current hr-HPV prevalence in developed countries (for ages 25 and older the split would be around 10% HPV-positive and 90% HPV-negative).

Background

The rather low specificity of the HPV assay requires the triage testing of HPV-positive women. However, although cytology is currently considered an appropriate triage tool for high-risk (hr)-HPV-positive women, there is still room for improvement given its subjective nature and rather low reproducibility. Several attractive and alternative triage tools based on biomarker analysis have recently become available. Amongst these, dual-stain p16/ki67 cytology testing is highly promising and seems to perform better than cytology.^{1,2} Ideally, however, a triage test should be fully compatible with the advantages of the hr-HPV test in terms of both reproducibility and quality as well as sample flexibility, especially as the latter would allow its application to self-collected cervico-vaginal samples.

Given the fact that cervical cancer development is a long-term process resulting from the accumulation of (epi)genetic alterations in the host cell genome following a transforming HPV infection, identification of these additive events may yield promising disease-specific markers that can be used as a triage tool. We anticipated that those alterations that are functionally relevant in malignant transformation (thereby representing drivers rather than passengers) will most likely yield the most valuable triage markers.

Biomarker identification and verification

A combination of *in vitro* and *in vivo* analysis has led to the identification of a number of functionally relevant genes involved in HPV-mediated transformation, including candidate tumour suppressor genes silenced by promoter methylation. These latter genes are attractive candidate biomarkers since DNA methylation, in which a methyl group is covalently bound to cytosine in a CpG dinucleotide, can be detected with high sensitivity by (quantitative) methylation-specific Polymerase Chain Reaction (PCR) ((q)MSP). This not only offers the opportunity to detect gene methylation in cervical scrapings^{3,4} but also in self-collected cervico-vaginal lavage specimens.⁵ We and others have reported an increasing number of methylated (candidate) tumor suppressor genes as well as miRNAs in cervical (pre)cancers.^{3,4,6-8} However, silencing of only a few of them by pro-

motor methylation has been functionally linked to HPV-mediated transformation and cervical carcinogenesis. Besides hsa-miR124,⁷ these include the genes encoding T-lymphocyte Maturation Associated Protein (MAL) and Cell Adhesion Molecule 1 (CADM1; formerly referred to as TSLC1).^{4,9} Based on the distinct stages of onset of silencing of these genes during transformation,^{4,9} we hypothesised that methylation analysis of MAL and CADM1 may be complementary in terms of CIN3+ detection.

Application of four qMSPs, two each for CADM1 (M12/M18) and MAL (M1/M2), to 261 cervical tissue specimens indeed showed that the highest positivity rates for Cervical intraepithelial neoplasia (CIN)3 lesions (97%) and carcinomas (99%) were obtained by combining a single CADM1 marker with a single MAL marker.¹⁰

Clinical validation

We subsequently composed, trained and validated a CADM1/MAL-based qMSP marker panel to stratify hr-HPV-positive women for cervical intraepithelial neoplasia (CIN)3+. Training and validation of the markers were performed on two large, independent sets of hr-HPV-positive scrapes that were collected during population-based cervical screening studies. Application of the best CADM1/MAL panel revealed CIN3+ sensitivities ranging from 60.5% to 100%, with corresponding specificities ranging from 22.7% to 83.3%. Interestingly, the point-estimates of both cytology and cytology/HPV-16/-18 genotyping were equal to the values of the receiver-operating characteristics (ROC) curve of the methylation marker panel.¹¹

Conclusions

The CADM1/MAL methylation marker panel provides an objective triage tool that is at least as discriminatory for CIN3+ as cytology or cytology with HPV-16/-18 genotyping in hr-HPV-positive women. This opens up the possibility for complete cervical screening by objective, non-morphological molecular methods to be applied directly to self-collected samples.

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THE ESSENCE OF A HISTORICAL SCIENCE-BASED POLITICAL DECISION

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Prevention of cervical carcinoma can be achieved by preventive vaccination against HPV infection or by screening for premalignant disorders of the cervix. The Dutch screening program for the prevention of carcinoma of the cervix is well organized and is one of the best programs in the world. However, studies on the cytological history of women diagnosed with carcinoma of the cervix have shown that more than 50% had never been screened previously.^{1,2}

Prevention will possibly be improved by the introduction of HPV vaccination, which commenced in the Netherlands in 2009.³ All school-age girls are invited for this vaccination at the age of 12.

It is, however, important that screening for cervical abnormalities be maintained for several reasons. First, because vaccination protects against 70% of oncogenic HPV types, second because the participation rate in vaccination will never reach 100% (in fact it is much lower than expected) and finally because it will take between 15 and 30 years for the effects of preventive vaccination on the incidence of cancer to become clear.

Recent scientific results have revealed options for improving the screening program.

A persistent hr-HPV infection is the main risk factor for cervical cancer, and it has been found that screening based on the detection of HPV DNA in the sample instead of abnormal cells is a better predictor for high-grade CIN and cancer.^{4,5} Furthermore, use of the so-called thin-layer technique offers the possibility to store material for cytological investigation in the event of a positive HPV test.

The Dutch Council for Health Care has therefore proposed a change in the screening program: screening will start with an HPV test, and cytology will only be performed in the event of a positive hr-HPV result. Referral for colposcopy will take place in the event of a positive HPV test in combination with abnormal cytology. When cytology is normal at baseline and after six months, the HPV test will be repeated first after five years.

Overall, the number of invitations per women will drop from seven to five.

In addition, the commission proposes to send a repeat invitation to non-responders after six weeks. Those women who still do not respond to this second invitation will receive an HPV self-test, the validity of which has been proven in several pilot studies.^{6,7} It has been estimated that this set up will lead to a higher detection rate and a decrease in mortality due to carcinoma of the cervix.

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Background

Although population-based cytology screening in developed countries has led to a substantial reduction in the incidence and mortality of cervical cancer,¹ cytology screening has some limitations. Thus, the accuracy of cytology is variable, often resulting in many false-positive and false-negative smears, and the sensitivity for high-grade lesions varies between 50% and 70%.² Furthermore, to compensate for the low sensitivity of a single smear, frequent testing is mandatory for successful implementation. Large randomized screening trials have shown that testing for high-risk (hr)-HPV provides better protection against cervical (pre)-cancerous lesions than cytology and is therefore an attractive primary screening tool.³⁻⁵ However, hr-HPV testing has a four- to sixfold lower specificity than cytology as some transient infections are also detected, particularly if performed in younger age groups.^{2,6} In countries with organized cervical screening at intervals varying from three to five years, it is vital to control the number of colposcopies and thus to keep the costs within acceptable limits. hr-HPV-positive women should therefore be triaged. However, although several triage suggestions, including cytology, hr-HPV genotyping, hr-HPV type-specific persistence analysis and p16^{INK4a} immunostaining,^{2,7,8} have been made in the literature, no uniform algorithm has yet emerged. A recent population-based screening study in the Netherlands, however, has analyzed new triage strategies and reached some interesting conclusions.⁹

Triage strategies for hr-HPV-positive women

The above-mentioned study evaluated several triage strategies for hr-HPV-positive women as regards negative predictive value (NPV), positive predictive value (PPV), sensitivity, specificity, and colposcopy referral rate. A triage strategy was considered feasible if the NPV was equal to or exceeded a predefined threshold of 98%.¹⁰ Five screening strategies with a triage test at baseline *that did not require* a repeat sampling visit (i.e. using liquid-based specimens suitable for performing the triage test) were evaluated. Such a screening strategy would be attrac-

HOW SHOULD HPV-POSITIVE WOMEN BE TRIAGED IN POPULATION-BASED SCREENING?

tive for implementation as no follow-up is needed, thus meaning that loss to follow-up is not a problem. The NPV and population-based colposcopy referral rates for these strategies are shown in Table 1. Of these "one-visit" strategies, only triaging with cytology combined with HPV-16/-18/-31/-33/-45 genotyping met the NPV criterion of 98% (i.e. 98.9%). However, the corresponding high colposcopy referral rate in the total population (2.95%), which is almost three times higher

than that obtained with cytology triage alone (1.09%), is a major disadvantage as, in addition to the increase in costs, it also poses a large burden on the daily practice of gynecologists. By adding one repeat test at 6 or 12 months, this referral rate is reduced markedly. Consequently, nine strategies with baseline triage followed by one round of repeat testing at 12 months were evaluated. Four of these strategies are shown in Table 2. In these strategies, baseline triage consisted either of cytology

alone or of cytology combined with hr-HPV-16/-18 genotyping.

The most attractive strategy was reflex cytology at baseline followed by repeat cytology testing at 12 months, with an overall colposcopy referral rate of 1.70% and an NPV of 99.3%.

Loss to follow-up may, however, be a problem when implementing a screening strategy including a repeat test. Indeed, several studies have shown that attendance at repeat testing is poor, particularly after a cytologically normal test result.^{11,12} Appropriate communication strategies are therefore necessary to improve attendance at repeat testing.

In addition, the logistics of a triage and follow-up strategy in a national program should preferably be simple. Furthermore, to prevent anxiety among women, it is essential that the last triage test received before women are directed back to routine screening is negative. A repeat visit for cytology at only 6 or 12 months meets this requirement. Other options, such as HPV-16/-18 genotyping after one year, seem less straightforward as women who are hr-HPV-positive, but HPV-16/-18 negative at a repeat visit, may feel uncomfortable about their mixed test result even if their risk of a high-grade lesion is low enough for them to return to the next screening round.

In conclusion, the evaluation of triage strategies for hr-HPV-positive women in a population-based study in the Netherlands strongly points to the use of cytological testing at both baseline and 6 or 12 months. This is a feasible and simple triage strategy because it has a high NPV for CIN3+, modest colposcopy referral rate, and is easy to communicate to physicians and women. Accordingly, this triage strategy is preferred in the Netherlands.

Triage strategy	hr-HPV-positive women	Total screening population
	Endpoint CIN3+ NPV %(95%CI)	Colposcopy Referral rate %(95%CI)
Cytology	95.1 (93.0-96.7)	1.09 (0.96-1.22)
HPV-16/-18	93.4 (91.0-95.2)	1.65 (1.49-1.80)
HPV-16/-18/-31/-33/-45	95.1 (92.4-96.8)	2.58 (2.39-2.77)
Cytology & HPV-16/-18	97.1 (94.9-98.4)	2.21 (2.03-2.39)
Cytology & HPV-16/-18/-31/-33/-45	98.9 (97.6-99.5)	2.95 (2.75-3.17)

CI = confidence interval; NPV = negative predictive value

Table 1.

NPV and colposcopy referral rates for five baseline triage strategies for hrHPV-positive women, adjusted for non-attendance at repeat testing

Baseline triage test	Repeat test at 12 months	hr-HPV-positive women	Total screening population
		Endpoint CIN3+ NPV% (95%CI)	Colposcopy Referral rate %(95%CI)
Cytology	Cytology	99.3 (98.1-99.8)	1.70 (1.54-1.85)
Cytology	HPV type persistence	97.5 (95.2-98.7)	2.45 (2.26-2.64)
Cytology	Cytology & HPV16/18	99.5 (98.1-99.9)	2.31 (2.12-2.49)
Cytology & HPV-16/-18	Cytology	99.7 (98.4-99.9)	2.53 (2.34-2.73)

CI = confidence interval; NPV = negative predictive value

Table 2.

NPV and colposcopy referral rates for four triage strategies for hr-HPV-positive women based on baseline and one round of repeat testing, adjusted for non-attendance at repeat testing

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COSTS AND BENEFITS OF HPV SCREENING: ESSENTIAL COMPONENTS

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To maintain a low incidence of cervical cancer, the Dutch government spends about 30 million euros per year on a cervical cancer screening programme for women aged between 30 and 60 years. This programme is carried out under the responsibility of regional screening organisations that invite women for cytological testing at five-year intervals. Uptake of the programme is acceptable, although there is some room for improvement (in 2009, about two-thirds of the women responded to the screening invitation).

The costs involved in preventing cervical cancer have nearly doubled since 2009 because of the introduction of HPV vaccination. The Dutch Ministry of Health, Welfare, and Sports is therefore open to changes in the current screening programme only if costs do not increase further. Replacing cytological screening by primary screening via HPV testing is one of the most obvious proposals and has been studied by the Dutch Health Council. However, the decision whether or not to implement HPV screening is not clear-cut. HPV testing has a lower specificity than cytology and further triaging of HPV-positive women seems necessary in order to minimize excessive colposcopy referrals. Furthermore, the current costs of HPV testing are calculated to be higher than those of cytology. Of course,

the costs of HPV testing will drop when HPV DNA becomes the primary screening test.

To prepare an advice on the implementation of HPV screening, the Dutch Health Council requested the Erasmus Medical Centre Rotterdam and the VU University Medical Centre (VUMC) Amsterdam to carry out cost-effectiveness analyses on the basis of their models.^{1,2} The goal of these analyses was to weigh the predicted benefits of HPV screening on cancer morbidity and mortality against costs. Only screening strategies with 5 or 10 year intervals were admissible, and the analyses were carried out using a cost-based tariff for the HPV test of 33 euros (GP5+/6+-PCR test). Furthermore, HPV-positive women were referred for colposcopy only after abnormal cytology and dismissed from further procedures after two negative Pap smears. Such a triage/follow-up strategy warrants a high positive predictive value for high-grade cervical intraepithelial neoplasia (CIN).³ The results for primary screening via combined HPV and cytological testing were not included in the final report as the negative predictive values of HPV testing and combined HPV and cytology testing were similar in a large screening cohort,⁴ and earlier cost-effectiveness analyses showed unfavourable results for combined testing.²



SELF-SAMPLING IN CERVICAL SCREENING PROGRAMS: FROM CURRENT TO FUTURE PRACTICE

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Background

Cervical screening has relied on cytomorphological analysis of cervical smears, a procedure that requires the intervention of a medical practitioner to collect a sample, for decades. Some women find this procedure to be as uncomfortable, embarrassing, and inconvenient. In addition, it can conflict with personal beliefs and religion. These difficulties likely contribute to non-participation in screening programmes, thus suggesting the need for novel screening strategies to optimally prevent and fight cervical cancer.

The benefits and opportunities of self-sampling

With the introduction of hr-HPV testing,

alternative sampling tools for cervical screening, including self-collection of cells from the vagina or cervico-vagina, have been evaluated. A variety of collection devices, including brushes, Dacron or cotton swabs, tampons and lavage devices, have been evaluated for self-collection. In general, women report to prefer self-collection over clinician-collection, although some minor differences in woman's preference and device performance have been described. Reasons in favour of self-collection include time and place of sampling, privacy and ease of sampling. Given this preference, it is not surprising that offering self-sampling for hr-HPV testing attracts up to 30% of non-attendees, i.e., women who were reluctant

to participate in the regular screening program involving a clinician-taken smear, into the screening program.¹⁻⁵ In this context, offering self-sampling could also reduce loss to follow-up, for example during post-treatment monitoring of women treated for high-grade Cervical Intraepithelial neoplasia (CIN).

Although self-collected (cervico-) vaginal samples are not suitable for accurate cytological assessment, sufficient data have been collected to indicate that hr-HPV DNA testing on self-collected samples is highly concordant with that on clinician-collected samples for hr-HPV DNA detection.^{6,7}

The cost-effectiveness analyses from Erasmus Medical Centre and VU University Medical Center (VUMC) were consistent.⁴ Some key results from the VUMC analyses are presented in Figure 1. The VUMC model predicts that replacing five-yearly cytological screening by five-yearly HPV screening is cost-effective according to formal health-economic criteria and leads to a reduction in the number of cancer cases of about 20%.² Costs are predicted to increase moderately, which may be countered by extending the screening interval.⁴ In Figure 1, we present results for HPV screening with an extension of the interval to 10 years for HPV-negative women aged 40 years and older. The interval remains five years for HPV-positive women with negative cytology. If the interval is extended, HPV screening can be implemented in a budget-neutral way while the cancer incidence is still predicted to decrease for women aged 35-44 years. This illustrates that HPV screening is feasible from a health-economic perspective even when there is a large concern about the costs of screening and detection/treatment of CIN.

Some important issues have not been addressed in the cost-effectiveness analyses. HPV screening was assumed not to lead to a decrease in screening uptake per round. Although there is no reason to believe that HPV screening will be less well accepted by women than cytological screening, screening organisations will need to accurately inform women in order to ensure broad acceptance of the viral test and to minimize distress caused by a positive test result. Furthermore, the temporary costs of reallocating health resources were not included. It is unlikely that implementation of HPV screening will be suspended or halted because of capacity barriers, but authorities should be prepared for the demand for extra laboratory and health-care personnel.

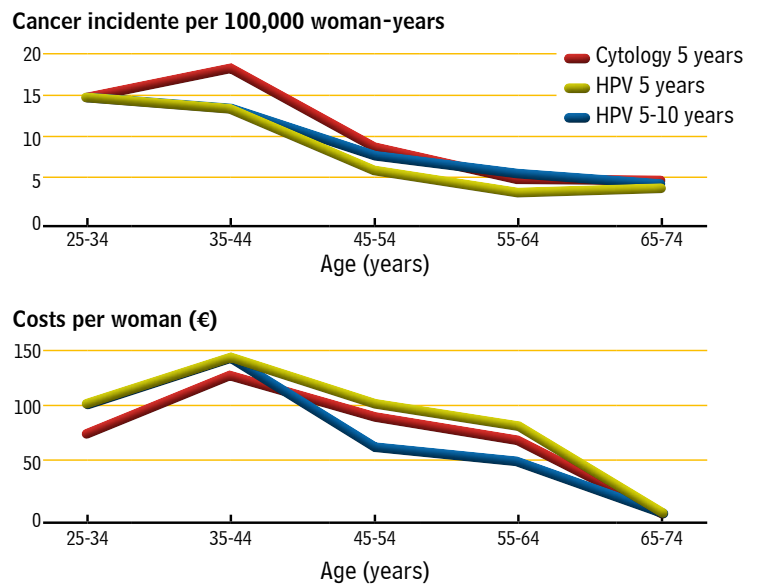


Figure 1. Cancer incidence (per 100,000 woman-years) and costs per woman predicted by the VUMC model. Screening strategies are: (a) five-yearly cytological screening between ages 30-60 years; (b) five-yearly HPV screening between ages 30-60 years; HPV-positive women are further tested by cytology; (c) 5-10 yearly HPV screening between ages 30-60 years; HPV-positive women are further tested by cytology; 10-year interval only for women 40 years and older who are HPV-negative on the primary HPV test.

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This most likely reflects the fact that viral DNA of an infected woman is sufficiently shed from the surface epithelium of the cervix to be detected in self-collected (cervico-)vaginal samples. Therefore, as hr-HPV testing will become the primary screening tool, self-sampling is likely to become an attractive alternative for screening, particularly with respect to targeting women who would otherwise be unable or unwilling to attend for a clinician-taken smear. Furthermore, self-sampling facilitates access to cervical screening of women from low-resource countries or isolated geographic localities lacking medical services.

Detection accuracy for high-grade CIN and cervical cancer (CIN2+ /CIN3+)

Various studies have shown that hr-HPV testing on self-sampled specimens is equally as, if not more, sensitive for CIN2+ as cytology on clinician-obtained cervical smears. Importantly, hr-HPV testing on self-samples can be equivalent to clinician samples in terms of sensitivity for CIN2+. This is supported by two recent

large studies among non-attendees in which a lavage device and brush-based device, respectively, were offered to non-attendees.^{2,5} In both studies, women with an Hybrid Capture 2 (HC2) (Qiagen Gaithersburg, Inc, MD, USA previously Digene Corp) hr-HPV-positive self-sample were triaged by cytology performed on a subsequent clinician-collected smear, and referred to colposcopy if cytology was positive (i.e., cytology reading as borderline or mild dyskeratosis or worse, equalling Abnormal squamous cells of undetermined significance (ASCUS)/ Low squamous intraepithelial lesions (LSIL), or, in case of normal cytology, if cytology and/or HPV was positive after one-year follow-up testing. Using this approach, a similar CIN2+ rate was found in women who were registered as non-responders but for whom it was the first screening round in their life (i.e., age 30-34 years), as in their age counterparts screened in the regular programme by hr-HPV test on a cervical scrape and similar cytology triage scheme.²

Conclusions

The data collected indicate that offering hr-HPV testing of self-sampled (cervico-) vaginal specimens to non-attendees of the regular screening program significantly increases screening attendance, produces hr-HPV test results that are in good agreement with those of clinician-taken scrapes in women with CIN2+ /CIN3+, and is effective in detecting CIN2+ /CIN3+.

Women who test hr-HPV-positive on their self-sample are currently advised to visit a clinician for a cervical smear, with a substantial risk of loss to follow-up. In the future this step is likely to be avoided by applying molecular triage markers directly on the self-collected samples. Taken together, the time has come to introduce self-sampling as an alternative tool for cervical screening by hr-HPV testing on clinician samples.



AUDIT STUDIES ON CERVICAL CARCINOMAS IN THE NETHERLANDS: WHAT DO THEY TELL US?

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Audit studies in the Netherlands try to answer the question of how many women are diagnosed with cervical cancer despite a well-organized screening programme.

The three Dutch audit studies performed since reorganisation of the screening programme in 1996 will be briefly discussed here (Table 1).

All these studies have obtained data from the national pathology and cytology database (PALGA), which registers all histology and cytology diagnoses in the Netherlands.¹ The investigators tracked down carcinoma cases from either PALGA or from a clinical setting and linked these cases, in silico, to their respective preceding screening histories.²⁻⁴ In trying to order all preceding screening histories into comprehensive results, the investigators faced limitations and had to make choices to circumvent them. First, PALGA has no link to the invitational database for call and recall, and information regarding the 'invitational status' of cervical cytology was only added to PALGA in 2006. To make a distinction between invitational screening smears and opportunistic screening or diagnostic work-up smears because of symptoms, investigators relied on the date of birth of women (as a proxy for invitation) or, after 2006, retrieved this information from PALGA. The second limitation of PALGA is a lack of information on clinical symptoms. This makes it difficult to distinguish opportunistic smears from diagnostic work-up smears. The latter can therefore easily be misinterpreted as screening smears. Finally, PALGA is an anonymised database, thus making linkage between preceding cytology history and carcinoma cases not 100% certain.

Because of these database limitations, the investigators handled different time-frames and used different definitions to distinguish 'regularly screened', 'underscreened' (i.e. no response to last invitation but smear(s) in previous round(s)) and 'unscreened' (i.e. no smear at all in the history). In this scenario, it is possible to make assumptions on the lower boundary to distinguish between 'screened' and not properly screened (un- or underscreened) women. For example, a screening test preceding the diagnosis of cancer by only about three weeks may be a true screening test but is more likely to be a diagnostic work-up test. One audit study used a one year preceding period as the lower boundary threshold to distinguish screening from work-up smears, whereas the other studies did not use lower boundaries (Table 1). The upper boundary used to distinguish screened from underscreened women is less controversial. The screening interval in the Netherlands is five years, and women are invited either prior to or after their birthday. In one study, women with a last smear taken >5 years preceding the diagnosis were considered under-screened, whereas the other studies used a threshold of ≥6 years (Table 1).

Although, given the use of different thresholds, comparisons

between the various studies should be made with great care, a clear time tendency becomes apparent. Thus, the audit results for carcinomas diagnosed prior to reorganisation of the programme (i.e. 1994-1998) suggest a less efficient invitational system in that time period, given the relatively high percentage of unscreened women, i.e. 67% (Table 1: second column), compared to 25% and 17%, respectively, in the later two studies (third and fourth column). On the other hand, the percentage of under-screened cases was higher (35% and 37%, respectively) in the more recent audit studies. Results for normal cytology preceding carcinoma within either regularly screened cases (first audit study) or the combination of under-screened and regularly screened women in the more recent studies were 64%, 42% and 39%, respectively. These values likely reflect an improvement in screening after reorganization of the programme. Introduction of hr-HPV screening will certainly lower this percentage further. Finally, two audit studies measured delay after abnormal cytology. This percentage increased from 18% in the period 1994-1998 to 39% in the period 2006-2007, which is a cause for concern.

	Audit 1994-1998 ² (N=1189)	Audit 1991-2008 ³ (N=269)
Age strata (years)	30;35-53;60 [#]	30-60
Threshold lower boundary (distinction screening from work-up smear)	None	None
Threshold upper boundary (distinction screened from under-screened women)	<6 years	≤5 years
Unscreened	797 (67%)	68 (25%)
Underscreened	108 (9%)	94 (35%)
Screened	284 (24%)	107 (40%)
Results cytology:		
Normal	183/284 [§] (64%)	85/201 [§] (42%)
≥BMD	101/284 (36%)	116/201 (58%)
Time delay after ≥BMD cytology	18%	Not available

[#] Screened cohorts 35-55 and 30-60 before and after reorganisation

[§] Data available for regularly screened (N=284) only

[§] Data available for regularly and underscreened combined only (N=201; N=180)

[^] Ibid plus three cytology results were inadequate. Data based on extrapolation

BMD: Borderline or mild dyskaryosis

Table 1.

Screening history of women diagnosed with cervical cancer in the periods 1994-1998, 1991-2008, and 2006-2007, respectively

In summary, audit studies on aggregate levels are important for evaluating screening programmes, but require clear definitions on cut-offs that should be agreed upon beforehand. **Ideally, a framework that facilitates generation of data for these audits should be in place prior to the start of any programme. In its absence, patient-centred audits should be undertaken on a regular basis.** It remains important that pathologists and gynaecologists check the history of any case of cervical carcinoma and take action if needed.

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BUILDING CONSENSUS IN THE NETHERLANDS: THE LOGISTICS OF CHANGE

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Summary

The Netherlands can boast a longstanding history of nationwide cervical cancer screening. Indeed, a full nationwide screening program was implemented in 1985 and fully restructured in 1996. Ten years later, in 2006, hr-HPV-testing became part of the screening programme for triaging women with smears reported as borderline or mild dyskaryosis (BMD) for the first time. We are currently standing on the brink of a decision by the Dutch independent Health Council to advise the Minister of Health, Welfare and Sport on the consequences of HPV vaccination for cervical cancer screening and the full implementation of primary HPV screening instead of cytological screening.

Getting screening implemented

Although screening undoubtedly plays a greater or lesser role in the reduction of incidence and mortality of cervical cancer, no organized screening program is available in the majority of European countries. The Netherlands, together with the Nordic countries, the United Kingdom and certain parts of Italy, have been at the forefront of cervical screening and have provided the evidence for its efficacy.¹ Despite the limitations recognized in several early pilot studies (mainly high false-negative rates and low attendance), it was decided in 1985 to offer a cervical cytology smear test to all women between the ages of 35 and 53 every three years.

In an effort to improve the cost-effectiveness of screening, i.e. limit the costs of the screening program, it was politically decided that every Dutch woman would be allowed seven smear tests in her lifetime. However, it was left to the discretion of the professionals, who came together in the Coordinating Committee for Cervical Cancer Screening (CoComBa), to match this requirement with a suitable screening algorithm. Subsequently, this new algorithm of screening at a five-year interval between the ages of 30 and 60 became part of a fully restructured screening program, in which follow-up, administration, financial arrangements and guidelines were also changed as of 1996.²

Screening was initially carried out by the regional health authorities or by the general physicians, supervised by the regional comprehensive cancer centres. More recently, however, the organization of, and responsibility for, screening has been placed in the hands of the National Institute for Public Health and the Environment (RIVM).

Efficacy

The most immediate effect of these changes was an increase in coverage and compliance and a decrease in opportunistic screening and repeat smears after 1996.³ However, even under these well organized conditions, cervical cancer screening remains marginally effective and therefore under the scrutiny of those for and against population screening.⁴ At best, 60% of cases of cervical cancer in the Netherlands will have been prevented by screening.⁵ Although screening coverage in the Netherlands is reported to be as high as 77%, the average response rate to calls for screening is 65%.⁶

Future

Whether or not cervical cancer screening, with all its limitations, will be changed remains mainly a political decision. The costs and logistical challenges of hr-HPV-based screening will certainly be taken into account by those in government and responsible for public health in their decision whether or not to follow the advice produced by the Dutch independent Health Council, which has now advised the Minister of Health how to proceed. Strangely enough, the national societies of the various professions involved in caring for cervical cancer patients (general physicians, screening organizations, gynaecologists, pathologists, radiation oncologists and medical oncologists) are no longer involved in the decision process, as they were during the time that CoComBa existed. The independent Health Council consists of experts and specialists who have been invited on the basis of their expertise and not as representatives of the professional organizations that perform screening and deliver care for those identified to be at high risk for cervical cancer. This choice has apparently been made to prevent conflicts with the political interests of the different professional organisations. As such, building a consensus in the Netherlands is an independent process towards consensus amongst individuals who are experts in their field, not amongst professional organizations.

Audit 2006-2007⁴ (N=217)

29-63

<1 year

<6 years

37 (17%)

81 (37%)

99 (46%)

69/177[^] (39%)
108/177 (61%)

39%

In 2006 the Dutch Society of Pathologists decided to add reflex high-risk (hr)-HPV-testing to follow-up smears of women with smears reported as BMD in order to reduce the recall rate. However, the guidelines do not make hr-HPV-testing compulsory, and many cytological laboratories still refrain from hr-HPV-testing for economic reasons or as a result of their unfamiliarity with molecular tests.

References: 1. Arbyn M *et al.* *Eur J Cancer* 2009;45:2671-8. 2. Rebolj van Ballegooijen M, Berkers LM, Habbema D. *Int J Cancer* 2007;120:806-12. 3. Berkers LM *et al.* *Ned Tijdschr Geneesk* 2007;151:1288-94. 4. van der Graaf Y. *Ned Tijdschr Geneesk* 2002 ;146:1569-71. 5. Bos AB *et al.* *Int J Cancer* 2006;119:2372-5. 6. Bulk S *et al.* *J Clin Pathol* 2004;57:388-93.



MOLECULAR HPV DETECTION ASSAYS: THE DUTCH CHOICE

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Background

With the recommendation of the Dutch Health council to switch from cytology to HPV detection for primary cervical screening, the important issue now is to use a valid HPV test for screening purposes. This is particularly relevant since previous studies have shown that various HPV tests display large differences in terms of clinical sensitivity and/or specificity for Cervical Intraepithelial neoplasia (CIN)2+/CIN3+. For screening purposes, an optimal balance between these two parameters is essential to avoid unnecessary follow-up procedures for women with transient high-risk (hr)-HPV infections, whilst at the same time ensuring high sensitivity and negative predictive value for CIN2+/CIN3+. This consideration has led to the formulation of European guidelines for HPV test requirements for primary cervical screening and validation guidelines

for candidate HPV tests.¹ The "Molecular Diagnostics in Pathology" working group of the Dutch Society for Pathology has already adopted these guidelines to set standards for the HPV tests to be used for triage purposes and has indicated that it will use the same criteria when considering HPV tests for primary screening.²

HPV detection assay requirements for screening

In essence, the HPV tests to be used in cervical screening should be clinically equivalent in terms of sensitivity and specificity for CIN2+ to assays such as Hybrid Capture 2[®] (HC2) (Qiagen Gaithersburg, Inc, MD, USA previously Digene Corp) and GP5+/6+-PCR-EIA, both of which have been clinically validated in large longitudinal screening trials.³⁻⁵ This can be determined in a cross-sectional clinical accuracy study on cervical scrapings collected from screening cohorts,

wherein candidate tests should prove to be non-inferior to Hybrid Capture 2[®] (HC2) (Qiagen Gaithersburg, Inc, MD, USA previously Digene Corp) or GP5+/6+-PCR according to formulated criteria for test requirements outlined in the validation guidelines.¹ Several novel hr-HPV detection assays have currently been found to fulfil the clinical sensitivity and specificity criteria.⁶⁻⁸ In addition, the assays should demonstrate a sufficiently high intra- and inter-laboratory reproducibility.¹

Expected procedure in the Netherlands

The Health council considers the HC2 and GP5+/6+-PCR-EIA assays to be clinically validated and therefore suitable for primary HPV screening. Other HPV tests need to demonstrate compliance with the guidelines for a clinically validated screening test before approval by the Health Council is obtained for their use in the screening program.

References: 1. Meijer CJ *et al.* Int J Cancer 2009;124(3):516-20. 2. ([http://www.pathology.nl/nvvp/nvvpcoms3.nsf/uploads/E4375B04810F9D78C12577AD004EDB81/\\$FILE/WMDP%20Richtlijn%20Gebruik%20moleculaire%20HPV%20testen%20in%20het%20BVO%2022062010%20def.pdf](http://www.pathology.nl/nvvp/nvvpcoms3.nsf/uploads/E4375B04810F9D78C12577AD004EDB81/$FILE/WMDP%20Richtlijn%20Gebruik%20moleculaire%20HPV%20testen%20in%20het%20BVO%2022062010%20def.pdf)) 3. Mayrand MH *et al.* N Engl J Med 2007;357(16):1579-88. 4. Naucler P *et al.* N Engl J Med 2007;357(16):1589-97. 5. Bulkman N *et al.* Lancet 2007;370(9601):1764-72. 6. Carozzi FM *et al.* J Clin Microbiol 2011; PMID 21325553. 7. Hesselink AT *et al.* J Clin Microbiol 2010;48(3):797-801. 8. Snijders PJ, Heideman DA, Meijer CJ. APMIS 2010;118(6-7):520-528.



THE SELF-SAMPLING DEVICE

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The self-sampling devices tested in our large studies are a *lavage* device (Delphi Screener[™], Delphi-Bioscience^{BV} Figure 1),¹ which is designed to rinse the upper vagina and the cervix, and a brush device (VibaBrush[®], Rovers Medical devices; Figure 2),² which merely scrapes the vaginal region. hr-HPV testing on specimens self-collected by either device showed good Cervical Intraepithelial Neoplasia (CIN)2+ detection accuracy. From these studies it appeared that the highest yield and fraction of cervical cell material was obtained with the Delphi Screener[™], thus making this sampled material more suited for triage testing with molecular markers. Second generations of both devices are currently being manufactured.



Figure 1. Delphi-Screener[™] (Delphi-Bioscience), Scherpenzeel, the Netherlands



Figure 2. VibaBrush[®] (Rovers) Medical devices Av, Oss, the Netherlands

References: 1. Gök M *et al.* BMJ 2010;340:c1040. 2. Gök M *et al.* Int J Cancer 2011, in press



CRITICAL REFERENCES FROM THE NETHERLANDS

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²Dept of Pathology, VU University Medical Center, Amsterdam, The Netherlands.

Key findings of some landmark papers from the Netherlands are summarized below.

HUMAN PAPILLOMAVIRUS DNA TESTING FOR THE DETECTION OF CERVICAL INTRAEPITHELIAL NEOPLASIA GRADE 3 AND CANCER: 5-YEAR FOLLOW-UP OF A RANDOMISED CONTROLLED IMPLEMENTATION TRIAL

Bulkmans N, Berkhof J, Rozendaal L, van Kemenade F, Boeke A, Bulk S, Voorhorst F, Verheijen R, van Groningen K, Boon M, Ruitinga W, van Ballegooijen M, et al. *Lancet* 2007 Oct 3;370(9601):1764-72.

This paper described follow-up results for one of the first randomized-controlled trials to compare the effectiveness of high-risk (hr)-HPV screening with screening by cytology over two screening rounds. Women who participated in the regular cervical screening programme in the Netherlands were randomly assigned to combined cytological/hr-HPV DNA testing or to conventional cytological testing only. After five years, combined cytological/hr-HPV DNA testing was performed in both groups. A total of 8575 women in the intervention group and 8580 in the control group were followed up for sufficient time (≥ 6.5 years). Cervical intraepithelial neoplasia of grade 3 or more (CIN)3+ lesions were detected at baseline in the intervention group than in the control group (70% increase; 95% Confidence Interval (CI): 15-151; $p=0.007$). The number of CIN3+ lesions detected in the subsequent round was lower in the intervention group than in the control group (55% decrease; 95% Confidence Interval (CI): 28-72; $p=0.001$). The number of CIN3+ lesions over the two rounds did not differ between groups. Thus, implementation of HPV DNA testing in cervical screening leads to earlier detection of CIN3+ lesions, which may permit an extension of the screening interval.

HPV TESTING ON SELF COLLECTED CERVICOVAGINAL LAVAGE SPECIMENS AS SCREENING METHOD FOR WOMEN WHO DO NOT ATTEND CERVICAL SCREENING: COHORT STUDY

Gök M, Heideman DA, van Kemenade FJ, Berkhof J, Rozendaal L, Spruyt JW, Voorhorst F, Beliën JA, Babovic M, Snijders PJ, Meijer CJ. *BMJ*. 2010 Mar 11;340:c1040.

This paper describes the Prevention by offering hr-HPV testing on self-sounded cervicovaginal specimens trial (PROTECT) study, which was initiated to determine whether offering self-sampling of cervicovaginal material for hr-HPV testing is an effective screening method for women who do not attend regular cervical screening programmes (i.e. non-attendees). A total of 27,792 non-attendees (not responding to two invitations) were asked to use a self-sampler to collect a cervicovaginal sample for hr-HPV testing (self-sampling group). Those with an hr-HPV-positive self-sample underwent cytology triage at the Genral Primer (GP) level. The remaining 281 women received a second re-invitation for conventional cytology (control group). The compliance rate in the self-sampling group was significantly higher than in the control group (adjusted 27.5% versus 16.6%, $P<0.001$). The number of CIN2+ and CIN3+ lesions detected in self-sampling responders was 99 (1.3%) and 76 (1.0%), respectively. Thus, offering self-sampling for hr-HPV testing to non-attendees is a feasible and effective method of increasing coverage in a screening programme.

THE USE OF GENERAL PRIMERS GP5 AND GP6 ELONGATED AT THEIR 3' ENDS WITH ADJACENT HIGHLY CONSERVED SEQUENCES IMPROVES HUMAN PAPILLOMAVIRUS DETECTION BY PCR

de Roda Husman AM, Walboomers JM, van den Brule AJ, Meijer CJ, Snijders PJ. *J Gen Virol*. 1995 Apr;76 (Pt 4):1057-62.

This paper described the development of a second-generation consensus Polymerase Chain Reaction (PCR)-based test for the detection of a broad spectrum of HPV types in one reaction. This test was based on the first-generation GP5/6-PCR, which targets highly conserved sequences within the L1 region of the HPV genome. Compared to GP5/6-PCR, the GP5+/6+-PCR method provided an increased detection level, particularly of less common HPV types. It subsequently became the method of choice for many epidemiological and clinical studies and, together with Hybrid Capture 2[®] (HC2) (Qiagen Gaithersburg, Inc, MD, USA previously Digene Corp), is acknowledged to be clinically validated for screening purposes.

HUMAN PAPILLOMAVIRUS IS A NECESSARY CAUSE OF INVASIVE CERVICAL CANCER WORLDWIDE

Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Muñoz N. *J Pathol*. 1999 Sep;189(1):12-9.

Using a comprehensive HPV PCR approach, this study focused on a large series of cervical cancer specimens that were previously scored as HPV-negative using the L1-based MY09/11 consensus PCR in a worldwide case study. It was reasoned that the failure to detect HPV in these cases could be the result of sample inadequacy or integration events affecting the L1 gene. After combining all HPV testing data and excluding inadequate specimens, the worldwide HPV prevalence in cervical carcinoma was 99.7%. It was therefore concluded that hr-HPV is a necessary cause of invasive cervical cancer worldwide.

RELATION OF HUMAN PAPILLOMAVIRUS STATUS TO CERVICAL LESIONS AND CONSEQUENCES FOR CERVICAL-CANCER SCREENING: A PROSPECTIVE STUDY

Nobbenhuis MA, Walboomers JM, Helmerhorst TJ, Rozendaal L, Remmink AJ, Risse EK, van der Linden HC, Voorhorst FJ, Kenemans P, Meijer CJ. *Lancet*. 1999 Jul 3;354(9172):20-5.

This study described the first of a series of prospective studies conducted in the Netherlands to study the relationship between hr-HPV presence and development, persistence and progression of CIN lesions in women. A total of 353 women referred to their gynaecologist because of abnormal smears were enrolled and monitored by cytology, colposcopy and hr-HPV testing by GP5+/6+-PCR every 3-4 months during a median follow-up period of 33 months. Of 103 women with end histology CIN3, 98 (95%) had persistent hr-HPV infection from baseline. It was concluded that persistent infection with hr-HPV is necessary for the development and maintenance of CIN3.



INTERNATIONAL AGENDA

Helsinki, Finland

12th January 2012

Human Papillomavirus Vaccination: Safety, Sound, Efficacy and Public Health Effectiveness.

Venue: Helsinki Exhibition and Convention Centre
E-mail: hpsymposium2012@gmail.com
Web: www.rokotiitus.net/english.html

San Diego, California, USA

25th-26th January 2012

Cancer Immunotherapy 2012

Venue: Hilton San Diego Gaslamp Quarter
E-mail: info@iqpc.com
Web: www.cancerimmunotherapyevent.com

Rome, Italy

19th-21st April 2012

XI International Workshop of Lower Genital Tract Pathology -Cervical Cancer a Global War

Venue: NH Vittorio Veneto
E-mail: info@triumphgroup.it
Web: www.triumphgroup.it

Paris, France

26th-30th May 2013

18th International Congress of Cytology

Venue: Palais des Congrès
E-mail: exh@cytologyparis2013.com
Web: www.cytologyparis2013.com

Barcelona, Spain

7th-10th July 2012

22nd Biennial Congress of European Association for cancer Research

Venue: Barcelona International Convention Centre
E-mail: eacr@nottingham.ac.uk
Web: www.eacr22.eacr.org/index.php

Hong Kong, China

13th-15th July 2012

AOGIN 2012

Venue: Cheung Kung Hai Conference Centre, University of Hong Kong
E-mail: aogin2012@pctourshk.com
Web: www.aogin2012hk.org

Singapur, Singapur

19th-22nd July 2012

16th World Congress on Controversies in Obstetrics, Gynecology and Infertility (COGI)

Venue: Fairmont Singapore & Swissôtel The Stamford
E-mail: cogi16@congressmed.com
Web: www.congressmed.com/cogi16/

Rome, Italy

7th -12th October 2012

FIGO 2012

Venue: Fiera di Roma
E-mail: figo2012secretariat@triumphgroup.it
Web: www.figo2012.org

Lisboa, Portugal

8th-11th November 2012

17th World Congress on Controversies in Obstetrics, Gynecology & Infertility (COGI)

E-mail: cogi@congressmed.com
Web: www.congressmed.com/lisbon2012/

San Juan, Puerto Rico

30th Nov 2012 - 6th Dec 2012

28th International Papillomavirus Conference & Clinical and Public Health Workshop

Venue: Puerto Rico Convention Centre
E-mail: events@hbtravelpr.com
Web: hpv2012pr.org

Praga, Czech Republic

5th-7th September 2013

6th European Congress of the European Federation for Colposcopy

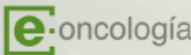
Venue: Prague Congress Centre
E-mail: Skrivanek@g-centrum.cz
Web: www.e-f-c.org

Seattle, Washington, USA


20th-25th August 2014

The 29th International Papillomavirus Conference

E-mail: HPV2014@ConferenceSolutionsInc.com
Web: www.hpv2014.org



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