1 INTRODUCTION

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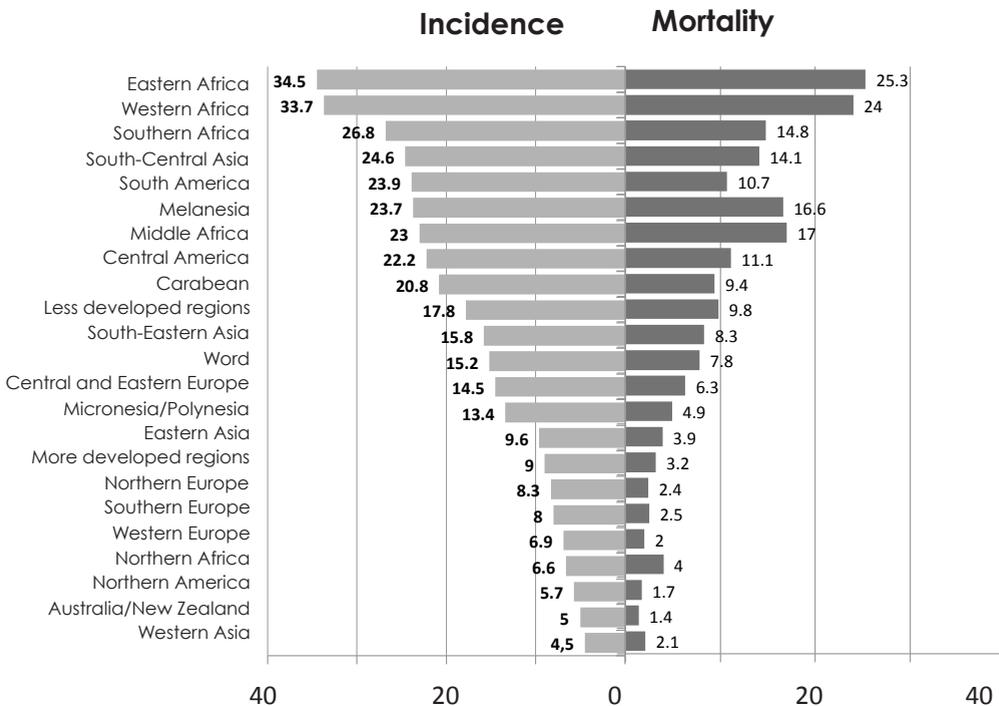
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1. Cervical cancer

Cervical cancer is an important public health problem. With 530,0000 new cases and 275,000 deaths per year, cervical cancer is the third most common malignancy in women worldwide.[1;2] Cervical cancer affects relatively young women, mostly under the age of 45.[2] Furthermore, a disproportionally high incidence is observed in developing countries, especially in Africa, South Asia, and parts of Latin America, with incidence rates >20 per 100,000 women.[1] The lowest rates are found in Western Asia, Australia, New Zealand, North America, and Western Europe (Figure 1). In the Netherlands in 2010, cervical cancer was newly diagnosed in 718 women (age-standardised incidence rate of 6.0/100,000) and 205 cervical cancer deaths were observed (age-standardised mortality rate of 1.3/100,000).[3]

	Incidence	Mortality
Eastern Africa	34.5	25.3
Western Africa	33.7	24
Southern Africa	26.8	14.8
South-Central Asia	24.6	14.1
South America	23.9	10.7
Melanesia	23.7	16.6
Middle Africa	23	17
Central America	22.2	11.1
Carabean	20.8	9.4
Less developed regions	17.8	9.8
South-Eastern Asia	15.8	8.3
Word	15.2	7.8
Central and Eastern Europe	14.5	6.3
Micronesia/Polynesia	13.4	4.9
Eastern Asia	9.6	3.9
More developed regions	9	3.2
Northern Europe	8.3	2.4
Southern Europe	8	2.5
Western Europe	6.9	2
Northern Africa	6.6	4
Northern America	5.7	1.7
Australia/New Zealand	5	1.4
Western Asia	4,5	2.1

Figure 1 World Standard cervical cancer incidence and mortality rates per country (rate per 100,000).[1]

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1.1 Precursor lesions of cervical cancer

According to the classic concept cervical cancer usually arises in the cervical transformation zone. The transformation zone consists of metaplastic squamous epithelium that is located at the site where the squamous epithelial cells of the ectocervix (outer part of the cervix) meet the glandular columnar epithelial cells of the endocervix (inner part of the cervix). The position of the transformation zone is dynamic, shifting outwards during puberty, and, over the following years, gradually shifting towards, and into, the endocervical canal as squamous epithelium replaces the glandular epithelium. However, recent collected data supports the concept that cervical cancer and its high-grade precursor lesion develops from a discrete population of ectoendocervical squamocolumnar junctional cells, rather than cells from the transformation zone.[4] Apparently, these cells are more susceptible to the human papillomarvirus (HPV) mediated transformation and therefore prone to cervical cancer development.

Cervical cancer can be classified into different histological subtypes, of which squamous cell carcinoma is the most common one (accounting for about 80% of all cervical cancers). The second most common type is adenocarcinoma, accounting for approximately 15% of cervical cancers. Very rarely, other types, such as neuro-endocrine carcinomas and clear-cell carcinomas, are diagnosed.

Cervical squamous cell carcinomas develop through premalignant precursor lesions called cervical intraepithelial neoplasia (CIN). CIN lesions are classified into three groups: mild (CIN1), moderate (CIN2), or severe (CIN3, including carcinoma in situ) lesions, depending on the extent of replacement of the epithelial lining by atypical cells. In CIN1, which represents productive infections (see 2.2), up to one-third of the cells of the lower epithelial layer is replaced by atypical cells. In CIN3, the most advanced precursor stage representing a transforming HPV infection (see 2.2), from two-thirds up to the whole epithelial layer consists of atypical cells (Figure 2). Moreover, from CIN1 to CIN3, the cells become more atypical. Lesions are graded as carcinoma when atypical cells pass the basal layer (invasion). Whether cervical cancer develops through consecutive CIN1, 2 and 3 is under debate, since CIN2/3 lesions may develop rapidly following a high-risk HPV (hrHPV) infection, leaving only a very limited time frame for a preceding CIN1 lesion.[5;6] The premalignant lesions can regress, persist, or progress. Lesions graded as CIN1 display a high regression rate, whereas CIN3 has the lowest regression rate. CIN2 lesions, a subset of which represents productive infections, have an estimated risk of cervical cancer of about 40%.[7;8] CIN3 lesions have the highest risk of about 50% to progress to invasive cervical cancer.[8-10] Regression of CIN lesions is always associated with hrHPV clearance.[11]

Together, CIN2 and CIN3 lesions are referred to as high-grade CIN. As mentioned before, the majority of the high-grade lesions would never progress to cancer in absence of treatment.[8] However, at present it is not possible to distinguish morphological non-progressive lesions from progressive lesions. Therefore, women with high-grade lesions (CIN2+) are usually treated, resulting in a certain amount of overtreatment. These women are mainly treated through a loop electrosurgical excision procedure (LEEP).[12] CIN1 lesions are called low-grade CIN.[5;8] Women with these lesions are not treated but are recalled for a repeat cytology smear.[13]

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2. Human papillomavirus (HPV)

Persistent infection of the cervical epithelium with hrHPV is necessary for the development of cervical cancer.[14-17] HrHPV can be detected in almost all cervical squamous cell carcinomas[14] and in 94% to 100% of all adenocarcinomas.[18-20]

Papillomaviruses are small, double-stranded DNA viruses. So far, more than 100 different HPV types have been identified. HPV types that are associated with cervical cancer are classified as 'high-risk' (hr) or carcinogenic. Based on epidemiologic criteria at least a dozen high-risk HPV types have been identified (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59).[16;18;21] In addition, HPV types 26, 53, 66, 68, 73, and 82 are considered probably high-risk.[22] Low-risk (lr) HPV types such as HPV6 and HPV11 are associated with benign wart-like lesions. The risk for cervical cancer differs between hrHPV types. Women with an HPV16 infection have a significantly greater risk for developing CIN3 or cervical cancer (CIN3+) compared to those infected with other oncogenic types.[23-25] For example, in the POBASCAM trial, 18-month risk of CIN2+ in hrHPV-positive women was 13% after normal cytology and 34% after borderline or mild cytological abnormalities. If positive for HPV16, CIN2+ risks were 27% and 48%, respectively.[25] Likewise, HPV18-positive women have an increased risk for CIN3+, although to a lesser extent as HPV16.[24;25] Longitudinal studies with extensive genotyping have furthermore revealed that HPV31 and 33 conveyed increased risks of CIN2+ or CIN3+.[24-26] In fact, in a study reported by Naucler et al.[26] infection with HPV16, 31 and 33 conferred the highest risks of CIN2+ within 4 years of follow-up and were responsible for 33.1%, 18.3% and 7.7% of CIN2+ cases. Given these figures, it is not surprising that HPV16 causes more than half of the cervical cancers worldwide, followed by HPV18 (~16%), and HPV33 (~4%).[27] In addition, hrHPV infections can also cause cancer in other parts of the ano-genital tract, such as anal, vulvar, and penile cancer, as well as in the head and neck, particularly of oral and oropharyngeal origin.[22;28-31]

2.1 Prevalence of HPV infections

Genital HPV infections are relatively common among sexually active women.[32;33] It is estimated that about 80% of women are infected with a genital HPV infection during their lifetime.[33] Since hrHPV is transmitted through sexual contact,[34] the prevalence of hrHPV infection is highest in young women (ages 20-24) after starting sexual contact.[32;35;36]

In the Netherlands, the highest prevalence of about 24% is detected in 22-year-old women. The prevalence gradually declines by increasing age to under 3% in women older than 45 years.[37] Estimates of single-point prevalence of hrHPV infection among women participating in the screening programme in the Netherlands are between 4% and 5%.[38;39] Risk factors that are associated with acquiring an hrHPV infection are the number of sexual partners, the age at which sexual intercourse was initiated, and smoking.[17;40;41] In addition, contact with men who have promiscuous sexual behaviour is associated with increased risk for acquiring an hrHPV infection.[33;34]

Fortunately, most women clear the hrHPV infection within 1-2 years.[17;42] Persistent infection with hrHPV is a necessary but insufficient cause of cervical cancer.[14;18] Decreased efficiency of the immune system to clear an hrHPV infection and additional (epi) genetic transforming events are necessary for malignant progression.

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2.2 The viral life cycle and transforming infections

The viral life cycle is dependent on the differentiation of infected epithelium, and the virus uses the host replication machinery to generate progeny. The process is as follows: HPV infects the basal cells in the cervical epithelium and replicates in differentiating epithelial cells. Encapsidation takes place in the upper layers of the host epithelium and, finally, viruses are shed when the superficial epithelial cells die. HPV is thought to access the basal cells of squamous epithelium through small tears, known as microtraumas, in the mucosal surface (Figure 2). Besides the viral proteins E1 and E2, which are essential for viral replication, the virus relies entirely on the host cell DNA replication machinery for viral DNA synthesis. Viral E6 and E7 proteins are needed to create conditions that allow viral replication in differentiated, non-dividing epithelial host cells, in which the DNA replication machinery is normally not activated. The location of expression of the latter viral proteins is tightly regulated to the mid-zone of the epithelium. E6 binds to the human tumor-suppressor protein p53 and degrades it; p53 plays an important role in cell cycle control and apoptosis. E7 binds to the retinoblastoma protein (pRb) tumor suppressor protein and thereby disrupts the binding of pRB to E2F, which leads to S-phase entry through released E2F.[43;44] Thus, E6 and E7 stimulate a condition of DNA synthesis in the infected host cell, thereby supporting viral replication. Many HPV infections are productive infections, in which new viral particles are formed and released. Productive infections of the cervix may give rise to mild to moderate cellular abnormalities, histologically comparable with CIN1/CIN2, but not often to true pre-cancerous stages of cervical cancer.

Under conditions that the viral E6/E7 proteins are improperly expressed in the proliferating basal cells, they may stimulate viral transformation.[45;46] These so-called transforming infections are associated with histologically CIN2, CIN3 and cervical cancer (CIN2+). The mechanism underlying the deregulation of E6 and E7 expression is not completely understood. A possible explanation is integration of viral DNA in the host cell genome,[47;48] but methylation of E2 binding sites within the viral long control region (LCR) has also been suggested.[49] Deregulated expression of E6 and E7 in the basal cells will result in chromosomal instability and provides the driving force for further progression towards cancer. Free E2F resulting from interaction of E7 with pRb stimulates uncontrolled cell growth in proliferating cells.[50] With less p53 due to degradation by E6, the cell cannot cope with uncontrolled cell growth,[51] which triggers the development of genetic instability. As a consequence of deregulated E7 expression, the tumour suppressor p16INK4a is up-regulated. P16INK4a is a cyclin dependent kinase inhibitor that normally prevents inactivation of pRb by cyclin D1 and therefore induces cell cycle arrest at G1. However, in the presence of hrHPV E7 protein, upregulated p16INK4a has no effect since pRB is already inactivated by E7. The overexpression of the tumour suppressor p16INK4a throughout the cervical epithelium (i.e. diffuse immunostaining for p16INK4a) can be considered as a marker for lesions that harbour a transforming hrHPV infection.[52-54]

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3. High-risk HPV and cervical carcinogenesis

Cervical cancer develops through the following steps: hrHPV infection, hrHPV persistence, hrHPV transformation and development of precancerous lesions, and finally progression to invasive cervical cancer (Figure 2).[17] Backward steps do also occur, with hrHPV clearance and regression of cervical lesions to normality.

About 80% of all hrHPV infections are cleared and will not result in premalignant lesions.[5;40;55;56] The majority of the remaining 20% will develop into non-progressive low-grade CIN lesions characterized by a productive hrHPV infection. Only a small group of CIN lesions containing a transforming hrHPV infection are at risk of progression to cervical cancer. Next to viral persistence and a molecular switch into a transforming infection accumulation of additive (epi)genetic alterations is necessary for further progression towards invasive cancer.
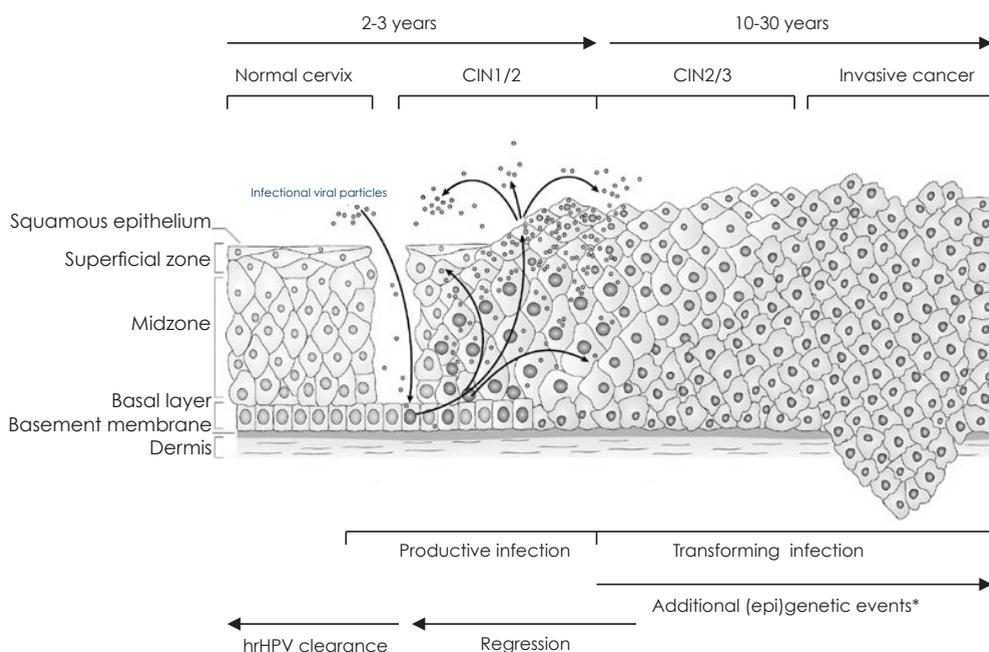
Figure 2 Schematic representation of cervical cancer development [adapted from [57]]. * Activation of oncogenes, loss of tumour suppressor gene function, p16INK4a overexpression, and chromosomal instability.

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4. Cervical cancer prevention

Two ways of cervical cancer prevention can be recognised: primary prevention and secondary prevention. Primary prevention is defined as an intervention aimed at taking the risk factor of cervical cancer, i.e. hrHPV infection, away in healthy women. Prophylactic vaccination with HPV16/18 L1 virus-like particles (VLPs), which was introduced in the Netherlands in 2009, is an example of primary prevention. Secondary prevention is an intervention to prevent cervical cancer by treatment of women who have subclinical (non-symptomatic) disease. Cervical cancer screening by detection of abnormal cells in cervical smears (also called a Pap smear) is an example of secondary cancer prevention.

4.1 Primary prevention by vaccination

Two prophylactic vaccines are available, a bivalent vaccine that protects against HPV16 and HPV18 (Cervarix®, GSK) and a quadrivalent vaccine that includes the low-risk types HPV6 and HPV11 as well (Gardasil®, Merck).[58-64] At present, vaccination does not eliminate the need for cervical cancer screening. Since the HPV vaccine protects against infection of HPV16 and HPV18, which together cause about 70% of cervical cancer cases,[58-60;65] screening will still be needed as a supplementary tool for the remaining 30%. Even with cross protection against non-vaccine HPV types, not more than 77% of cervical cancers will be prevented (HPV31 and 45 contribute to 7% of cervical cancer cases).[20] Moreover, by no means will all individuals of the target group come forward for vaccination. In the Netherlands, the HPV vaccine is given to HPV-negative (naïve) 12-year-old girls with an initial catch-up vaccination for 13- to 16-year-old girls, and an average of 56% coverage is observed.[66] As a result, for the majority of women, screening will remain the most important preventative strategy at least for the next 20 years.

4.2 Secondary prevention

Screening is an important cancer prevention tool. It is believed that nearly all cervical cancer-deaths could be prevented if women and their healthcare providers would fully adhere to screening recommendations and follow-up regimens.

Success of cervical cancer screening is due to three factors: first, cervical cancer has a long-lasting pre-invasive phase, allowing time for detection. The median time between precursor lesions and cervical cancer is estimated to be more than 10 years.[67;68] Second, cellular morphological changes on the cervix can be seen in the Pap smear and used for early diagnosis. Third, disease can be simply and effectively treated in the pre-invasive phase.

Tests used in primary cervical screening should fulfil certain requirements. *Clinical sensitivity, clinical specificity, positive predictive value (PPV), and negative predictive value (NPV)* are statistical measurements to express performance of a test. Clinical sensitivity is the probability that a test correctly classifies people with clinically meaningful disease at a preclinical stage as positive (e.g. the percentage of people with disease who are correctly identified as having the condition). Clinical specificity is the probability that a test classifies people without disease as negative (e.g. the percentage of healthy people who are correctly identified as not having the condition). Predictive

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values of test results depend on the prevalence of disease in the population. The positive predictive value (PPV) is the proportion of people with a positive test result who have the disease. The negative predictive value (NPV) is the proportion of people with a negative test result who do not have the disease.

Cervical screening should be performed in organized programmes with quality assurance at all levels and good information should be provided about the benefits and risks. Opportunistic screening activities are usually not acceptable as they may not achieve the potential benefits and may cause unnecessary negative side effects. The benefit of screening on the reduction of cervical cancer cases depends on the clinical sensitivity of the screening test, participation of invited women, and availability of adequate treatment and follow-up algorithms for women with abnormal test results.[17] The test used in screening needs to be robust and highly reliable and should therefore display high intra-laboratory reproducibility in time and inter-laboratory agreement. However, screening also has negative side effects. The negative effects of screening depend on the sensitivity (false negative tests) and specificity of the test (false positive tests, resulting in unnecessary treatment and distress about a positive test) and on the possible side effects of early treatment.

4.2.1 Cytology screening

The development of the Pap smear [69] has resulted in the implementation of cytology-based cervical cancer screening programmes.

For a cytological diagnosis, cells are scraped from the transformation zone of the cervix. These cells are placed on a glass slide, fixed, and coloured. The morphological changes of the cells are graded based on the subjective interpretation of the degree of abnormality. There are several commonly used classifications. In the Netherlands, the CISOE-A coding system is used (in Dutch KOPAC-B).[70;71] The American Bethesda

Table 1. Cytomorphological classification: the CISOE-A and Pap classification compared to Bethesda 2001 classification.

CISOE-A	S0, O0, E0	S1, O1 E1-2	S1, O1 E1-2	S2-3, O3, E3	S4, O4, E4	S5, O5, E5	S6, O6, E6	S7, E7	S8-9, O7-8, E9
Pap	Pap0	Pap1	Pap1	Pap2	Pap3a1	Pap3a2	Pap3b	Pap4	Pap5
Description	Inadequate	Normal	Normal	Border-line	Mild	Moderate	Severe	Carcinoma in Situ	Carcinoma
				Dyskaryosis					
Bethesda 2001 for	Un-satis-factory evaluation	NILM	Atrophy, NILM	ASC-US/ASC-H	ASC-H/LSIL	HSIL			SSC
				AGC	AGC favor neoplastic			AIS	AC

CISOE-A, C composition, I inflammation, S squamous epithelium, O Other abnormalities and endometrium, and E endo-cervical columnar epithelium; the acronym CISOE-A is KOPAC-B in Dutch. NHIL, negative for intra-epithelial lesions or malignancy; ASC-H, atypical squamous cells cannot exclude HSIL; ASC-US, atypical squamous cells of undetermined significance; AGC, atypical glandular cells; LSIL, low grade squamous intraepithelial lesion; HSIL, high grade squamous intraepithelial lesion; AIS, endocervical adenocarcinoma in situ; SCC, squamous cell carcinoma; AC, adenocarcinoma.

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classification is most commonly used in internationally published studies on cervical cancer screening.[72;73] The CISOE-A classification can be converted into the Bethesda system (Table 1).[74;75]

Until now, primary cervical cancer screening has been based on cytology. Several epidemiologic studies have shown that cytological screening has been successful in reducing the incidence and mortality of cervical cancer.[7;76-78] However, the cytology test has some limitations. The sensitivity of cytology for high-grade lesions is quite low (50-70%) and the performance is heterogeneous across populations [79]. Cervical high-grade lesions are missed by cytology due to sampling and detection failures.[80] To compensate for the low sensitivity of a single cytology test, frequent testing is necessary. Since the specificity of cytology is about 95%,[79;81] a substantial number of women with minor abnormalities, and who do not harbour any underlying high-grade disease, have unnecessary follow-up procedures. Moreover, adenocarcinoma and its precursors are frequently missed by cytology. In addition, cytology is a subjective test and labour intensive.[82]

Liquid-based cytology is a technique that has been developed to reduce variation in quality of cytology. With this technique, the cell sample is collected as in the conventional cytology test, but cells are immersed in a vial with preservation solution instead of spread onto a slide. In the laboratory, a concentrated monolayer of cells is prepared. The advantage of liquid-based cytology is that the slides are easier to interpret because the process reduces the amount of obscuring material, such as blood and leucocytes. The number of cytological preparations read by cytotechnicians per hour can be increased substantially using liquid-based cytology. Moreover, liquid-based specimens can be used for other ancillary tests, such as hrHPV tests and tests for other biomarkers. However, in contrast to what has been claimed in earlier publications, liquid-based cytology is not more sensitive for detecting high-grade cervical lesions than a conventional smear.[83-86] Disadvantages of liquid-based cytology include a lower number of cells and loss of structural integrity of cell groups, by which cohesion of neoplastic cells disappears. As a result, high-grade lesions and carcinomas might be missed.

4.2.2 Current screening in the Netherlands

In the Netherlands, cytological screening programmes were introduced in the 1970's. In three regions, Nijmegen, Utrecht, and Rotterdam, a pilot-screening programme was introduced. Nationwide screening was introduced in 1988 for women aged 35-54 with a 3-year interval. In 1996, the cytological screening programme was restructured; screening ages were extended to women aged 30-60 years, and the screening interval was widened to 5 years.[70] In addition, a cytological smear with cytomorphologic signs of inflammation and/or presence of specific microorganisms was no longer classified as Pap 2 (i.e. borderline dyskaryosis), but classified as within normal limits.[71;87] Moreover, a compact disc with microscopic examples of abnormal cytology was provided to the all the pathologists. This resulted in a decrease in the number of equivocal diagnoses from 11.3% to 2.6%.[74] Normal smears without endocervical cells were no longer repeated until a smear with endocervical cells was obtained, as there

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was doubt about the importance of these cells in cervical smears.[88] Furthermore, for quality control, the evaluation of the screening programme was improved. In 2006, negative triage with hrHPV testing on repeat smears after a borderline smear was incorporated.[89] Since 2008, both the conventional and the new liquid-based cytology technique have been accepted for the national screening programme.

In the present day, approximately 800,000 women are annually invited for cervical screening in the Netherlands.[90] In 2008, 550,000 women participated in the screening programme (67% of the invited population).[91] About 95% of the participating women had a normal smear. These women were invited for the next screening round after five years. In 1.9% of the women, the smear was inadequate, and these women needed to repeat the smear after six weeks. A smear with borderline or mild dyskaryosis (BMD) was observed in 2.5% of the women. Women with BMD were recalled for repeat cytology after 6 and 18 months and were referred for colposcopy if the repeat test result was positive (BMD or worse). This policy is used because only 5%-15% of women with BMD have or will develop high-grade cervical lesions.[92-94] Since 2006, an hrHPV test can be added to cytology for the first follow-up smear at 6 months after the baseline BMD smear.[89] This leads to a reduction in the number of repeat smears, as it is considered safe to refer women with normal cytology and a negative hrHPV test back to routine screening. In 0.7% of the women, a smear with moderate dyskaryosis or worse (>BMD) was present. These women were directly referred to the gynaecologist for colposcopy examination.[95;96]

The detection of cervical lesions classified as CIN1 or worse was 5.3 per 1,000-screened women. Of the women who were directly referred, 60% had CIN3+ and 17% had a CIN2 lesion.[96]

4.2.3. Screening in other European countries

Almost all European countries have cervical cancer screening. However, only seven have a national organized screening programme (Denmark, Finland, Hungary, the Netherlands, Slovenia, Sweden, and the United Kingdom).[97] Although all policies are mainly in line with the European recommendations (screening women every 3-5 years), there is a large variation in screening intensity. The number of smears taken in a lifetime varies between 7-16 per women, with the exception of Germany with a 1-year interval and over 50 smears taken in a lifetime.

The number of opportunistic smears taken in addition to screening varies strongly between countries. In the Netherlands, 77% of women aged 30-64 years had at least one smear in the past 5 years, whereas the response to the screening invitations was 65%.[91;98] Unlike in the Netherlands, in Finland there are many opportunistic smears taken in addition to screening. Annually, 460,000 smears are made while only 270,000 women per year are eligible for a smear.[99] An explanation for this might be that in the Netherlands the smears taken outside the screening programme are not reimbursed. In addition, in the Netherlands the general practitioner and assistant sample for cytology-screening tests, and they are well informed about the guidelines. Furthermore, many women in Finland have had a smear taken before the first invitation for cervical screening. Opportunistic smears add little to the effectiveness of the regular

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programme, and therefore decrease the cost-effectiveness of the screening activities.

Also age at which women are screened for the first time varies between countries. In Luxembourg, women are screened starting at the age of fifteen, in Belgium at the age of eighteen and in Germany at the age of twenty. Finland and the Netherlands have the highest age, i.e. thirty years, at which women are screened for the first time. With the upper age limit of 60 years, as also used in Denmark and Sweden.

Cervical cancer incidence rates vary widely across Europe. The highest incidence rates are seen in Eastern European countries. The lowest rates are seen in countries such as Italy, Switzerland, Spain, Finland, and the Netherlands.[1]

Despite the presence of a relatively effective screening programme in the Netherlands, cervical cancer continues to be a considerable public health problem.[100;101] The main reasons for missing cervical cancer cases despite a screening programme are a relatively low adherence to the screening programme (65% per round attendance rate of invited women) and a relatively high number of false-negative cytology tests (low sensitivity).[102;103] In addition, the follow-up of screen-positive women is not optimal. Therefore, over the last decades, efforts to improve screening have focused on increasing attendance and on development of alternative screening tests that are more sensitive than cytology.

5. Improvement of cervical cancer screening

Several trials have studied the value of hrHPV testing in cervical cancer screening programmes (Table 2). These studies have shown that hrHPV testing has a higher sensitivity for high-grade CIN than cytology testing.[79;104-108] However, the hrHPV test has a lower specificity because the hrHPV test detects a substantial number of women with transient hrHPV infections that will not lead to clinically meaningful lesions.[79;109] This may lead to over-referral for colposcopy and thus over-treatment.[107] As a result, women with a positive hrHPV test result should not be offered colposcopy immediately but should be further stratified by means of triage and repeat testing.

Testing for hrHPV can be used for various purposes: as a primary screening test, as triage for women with minor cytological abnormalities (BMD), and as follow-up test after treatment of pre-invasive lesions.[110]

A seemingly effective method of improving screening coverage is by sending self-sampling devices to women who do not respond to invitation for the screening programme.[111-113] About 30% of the non-attendees respond by returning their self-sampler.[112;114] Moreover, the detection of high-grade lesions in the self-sampling group was higher than in the regular screening programme.[112;114;115] Studies using interview surveys have shown that women prefer self-collection over physician-collection.[116-118] Time and place of sampling, privacy, and ease of sampling have been mentioned as advantages of self-sampling.

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5.1 HPV detection methods

A variety of hrHPV tests are currently available. HrHPV nucleic acids can be detected by target or signal amplification assays. Target amplification assays mainly comprise polymerase chain reaction (PCR)-based methods [119] and isothermal RNA amplification methods. PCR assays that are used include GP5+/6+,[120;121] PGMY09/11,[122] SPF10 [123] and the Roche Amplicor HPV test.[124] Examples of signal amplification assays are the Hybrid Capture 2 (HC2) test (Qiagen), Invader technology (Cervista HPV-HR Hologic), and in situ hybridisation (ISH) assays. In HC2, hybridisation of one of the RNA probes to hrHPV DNA is detected by biotin-labelled antibodies that recognize RNA/DNA hybrids following capture in streptavidin-coated microwell plates. The outcome of this test is expressed in relative light units per calibrator cut-off (RLU/CO).

When considering hrHPV tests, it is important to recognize the difference between analytical sensitivity and specificity versus clinical sensitivity and specificity. *Analytical sensitivity and specificity* refer to the detection of all hrHPV infections, including transient infections and those associated with high-grade lesions. Instead, *clinical sensitivity and specificity* point to the detection of only those hrHPV infections that are associated with clinically meaningful lesions. Ideally, the hrHPV test used in clinical settings should detect only women at risk for cervical cancer but not those with transient hrHPV infections.

The hrHPV tests GP5+/6+ PCR and HC2 have been used in large randomised controlled trials (Table 2) and have proven to perform well in cervical screening programmes. As such these assays are considered clinically validated. Both assays detect the hrHPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68, and, additionally, HPV66 is targeted by GP5+/6+ PCR and detected by HC2 as a result of cross-hybridisation.

However, many new hrHPV tests have been developed, and the clinical performance of these tests is mostly unknown. Therefore, standards for hrHPV test performance and characteristics in clinical practice have been formulated.[125] Using these guidelines, three hrHPV DNA tests (cobas® 4800, [Roche Molecular Systems Inc., Alameda, CA, USA], RealTime PCR [Abbott Molecular, Des Plaines, Il, USA] and Papillocheck® [Greiner Bio-One, Frickenhausen, Germany]) have proven to ful-fil the sensitivity and specificity criteria for cervical screening purposes and can be considered clinically validated following demonstration of a sufficient reproducibility.[126-129]

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Table 2: Studies with hrHPV testing and cytology in cervical cancer screening

Study	Study type	Age	Population	Primary screening test in intervention arm	Primary screening test in control arm
Hart study (Cuzick, 2003)	Multicentre screening study	30-60	10,358 women, United Kingdom	Conventional cytology + HC2	Conventional cytology + HC2
				Borderline cyto and hrHPV+ & normal cyto ->Referred for immediate colposcopy	Borderline cyto and hrHPV+ & normal cyto ->Follow-up with repeat hrHPV and cyto and colposcopy at 12 months
NTCC, Phase I (Ronco 2006, 2010)	RCT	25-60	45,174 women, Italy	LBC + HC2	Conventional cytology
NTCC, Phase II (Ronco 2008, 2010)	RCT	25-60	49,196 women, Italy	HC2	Conventional cytology
POBASCAM (Bulkmans 2007, Rijkaart 2012)	RCT	29-56 women,	40,105 cytology + the Netherlands	Conventional cytology GP5+/6+	Conventional
Swedescreen (Naucler 2007)	RCT	32-38	12,527 women, Sweden	Conventional cytology + GP5+/6+	Conventional cytology
CCCaST, (Mayrand 2007)	RCT	30-69	10,154 women, Canada	HC2	Conventional cytology
ARTISTIC (Kitchener 2009)	RCT	20-64	24,510 women, United Kingdom	LBC + HC2	LBC
India screening trial (Sankaranarayanan 2009)	RCT	30-59	131,746 women, India	HC2 vs. Conventional cytoloygyvs VIA	No screening
Finnish screening trial (Anttila 2011)	RCT	30-60	38,670 women, Finland	HC2 + Conventional cytology triage	Conventional cytology
VUSA-Screen (Rijkaart 2010)	Cohort study	30-60	48,088 women, the Netherlands	Conventional cytology+ HC2	Conventional cytology

LBC: liquid-based cytology, RCT: randomised controlled trial, VIA: visual inspection with acetic acid

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6. Outline of this thesis

Infection with hrHPV plays a causal role in the development of cervical cancer.[14;16] This has resulted in the development of hrHPV testing suitable for population-based screening. The goal of this thesis is to examine whether cervical screening can be improved by 1) hrHPV testing in triage of borderline or mild dyskaryotic cytology, and 2) hrHPV testing as a primary screening test. Other goals were to evaluate the potential clinical impact of hrHPV screening compared to cytology. In addition, we studied the optimal follow-up algorithm for hrHPV-positive women.

In most countries that screen for cervical cancer, including the Netherlands, women with borderline or mild dyskaryosis (BMD, similar to Pap2 and Pap3a1, in CISOE-A: S2-4, O3 E3-5) are recalled for repeat testing, and they are only referred for colposcopy if the cytological abnormality persists. This algorithm was developed since only 5%-15% of these women develop high-grade lesions (CIN2+). The disadvantage of this repeat testing algorithm is the loss of follow-up and distress for the women because of the uncertain diagnosis. In **Chapter 2**, we answer the question: is it feasible to use hrHPV triage for women with BMD? The VUSA-Screen study (**V**rije **U**niversiteit **M**edical Center **Sa**ltro laboratory population-based cervical **screen**ing) was designed to investigate the efficacy of additional testing for hrHPV in the cervical screening programme. In this study, women aged 30-60 years were offered hrHPV testing and cytology (intervention group) or cytology only (control group). In the intervention group, women with BMD and an hrHPV-positive test were immediately referred for colposcopy, whereas hrHPV-negative BMD women were advised to repeat cytology at 6 and 18 months and were referred for colposcopy when the repeat test result was positive. We compared hrHPV and cytology triage algorithms for women with BMD using the end point of histologically confirmed CIN3+, the number of repeat smears, the number of colposcopy referrals, and the medical costs.

Data from randomised controlled trials indicate that hrHPV screening has a higher sensitivity than cytology screening for detecting high-grade cervical lesions.[79;104-108;130] However, implementation of hrHPV testing as a primary screening instrument in cervical screening is still under debate. In **Chapter 3**, we present results from the VUSA-Screen study focusing on the following three implementation issues: 1) whether hrHPV testing in primary screening should be offered alone or as an adjunct to cytology, 2) how to manage hrHPV-positive women, and 3) the age of application of hrHPV-based screening.

In **Chapter 4**, we present the definitive 5-year results of the POBASCAM trial (**Po**pulation **Ba**sed **Sc**reening Study **Am**sterdam), a population-based, randomised controlled trial in which women were screened at baseline with either combined hrHPV testing and cytology or conventional cytology alone, from January 1999 to September 2002. At the subsequent screening round after 5 years, all women were screened with both hrHPV testing and cytology. Our main goal was to assess whether hrHPV testing decreases detection of CIN3+, CIN2+, and of cervical cancer in the second screening round. An additional goal was to assess the most appropriate age at which hrHPV testing should start. We also evaluated how detection of high-grade

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lesions in two screening rounds was associated with particular HPV genotypes.

Several studies have shown that hrHPV testing has a lower specificity than cytology screening for detecting high-grade cervical lesions.[79;104-108] The lower specificity has raised concerns that primary screening with hrHPV leads to increased referral rates for colposcopy and to overtreatment. Therefore, hrHPV-positive women should not be referred for colposcopy immediately. In **Chapter 5**, we used data from the intervention arm of the VUSA-Screen study to evaluate fourteen triage and repeat testing strategies for hrHPV-positive women. Another potentially cost-saving and simple option is to adapt the threshold for considering an hrHPV test as positive, i.e. increase the relative light unit/cut-off (RLU/CO) threshold of the Hybrid capture 2 (HC2) test. In **Chapter 6**, we studied the effect of hrHPV testing with higher HC2 thresholds on sensitivity, the colposcopy referral rate, and the false positive rate.

The results of the preceding chapters are discussed in **Chapter 7** and summarized in **Chapter 8**.

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