Screening in low resource settings, towards a world without cervical cancer

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Chapter 1

General introduction

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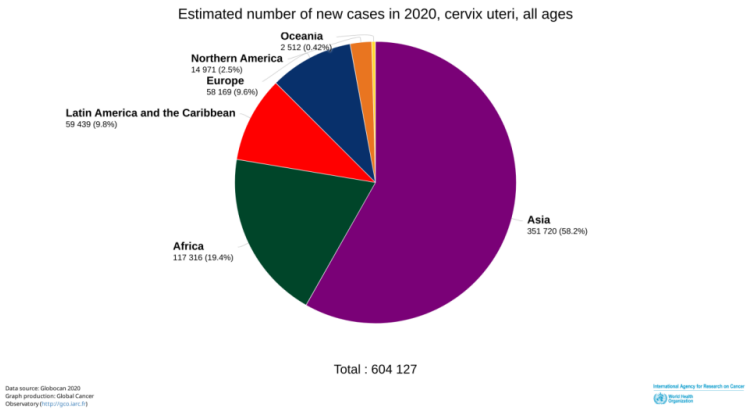
1. Cervical cancer epidemiology

Cervical cancer is the third most common cancer and the fourth leading cause of cancer death in females worldwide, with estimated 604,000 cases accounting for 9% of the total new cancer cases. Most cervical cancers are diagnosed in advanced stages with poor rates of survival, causing 342,000 deaths in 2020 which is 7.7% of the total cancer deaths among females worldwide (1).

In low- and middle-income countries, where approximately 90% of the worldwide new cervical cancer cases occur, cervical cancer is the second most common cancer after breast cancer. The highest incidence rates of cervical cancer are found in Asia, Africa, as well as in South America. Cervical cancer incidence increases from ages 35-40 years and reaches a maximum at 50-60 years of age. This means that women are affected in their reproductive age when they play central roles in their families (1, 2).



2. Etiology of cervical cancer

A persistent infection with high-risk human papillomavirus (hr-HPV) in the uterine cervix has been established as the cause of cervical intraepithelial dysplasia and cervical cancer (3-5). Human Papillomavirus is transmitted sexually and the lifetime risk for infection for women worldwide is 85%. Infection is most common in young women in their first decade of sexual activity, with the highest prevalence seen in women under 25 years of age (6-8).

HPV types are divided in low-risk non oncogenic types, causing benign genital lesions such as condylomata accuminata, and high-risk oncogenic types which can

cause premalignant and malignant lesions. Most cervical HPV infections are transient, asymptomatic, do not cause cytological abnormalities or cause low grade abdnormalities and are cleared within 1-2 years after exposure. When an infection with a high-risk HPV type persists, it can cause morphologic changes in the epithelium of the cervix. Premalignant lesions are mostly established within 5-10 years after an HPV infection. Generally, the duration of the whole process from HPV infection to invasive cancer has been estimated to take between 12-15 years. An alternative concept finds increasing support that clinically relevant lesions may rapidly arise within 2-3 years following infection (9-14).

The lifetime number of sexual partners, sexual behavior of women and their partners and the age at which sexual intercourse was initiated are associated with increased risk for acquiring a high-risk HPV infection (33).

Factors that increase the risk of persistence of the HPV infection include smoking, use of oral contraceptives for more than 5 years, high parity and previous exposure to other sexually transmitted diseases. Women exposed to the human immunodeficiency virus (HIV) are at high risk for persistence of HPV infection, and progression of premalignant lesions to cervical cancer due to an impaired cell mediated immune system which decreases viral clearance (15).

3. Human Papillomavirus (HPV)

Human papillomaviruses are from the papillomaviridae family. The HPV genome is a circular double stranded DNA and about 8,000 nucleotides. The HPV genome contains a non-coding long control region (LCR) and 8 open reading frames (ORFs) existing of 2 late genes and of 6 early genes. The late genes, L1 and L2 encode and compose the viral capsid. Of the 6 early genes, E1 and E2 modulate transcription and replication, E4 is involved in maturation and release of papillomavirus particles, E5 changes intracellular signaling and E6 and E7 modulate the transformation resulting in genetic instability and trigger a carcinogenic process (6, 16).

The process of the development of cervical cancer starts with a high-risk HPV infection at the cervical squamous columnar junction (SCJ). At the SCJ squamous cells from the ectocervix meet glandular cells from the endocervix. The area is dynamic as during lifetime, under the influence of hormones, it migrates onto the ectocervix and back into the endocervix creating the transformation zone (TZ). Here, the tissue is susceptible to the carcinogenic potential of HPV infections when the infections reach the basal cells of the epithelium through small tears in the mucosa.

The infected cells divide and migrate towards the epithelial surface where they shed, and where the virus can than initiate a new infection. The viral genome of high-risk HPVs frequently integrates into the cellular genome. This integration has been proposed as a mechanism of high grade squamous intra-epithelial lesions (HSIL) progression to invasive cancer (6, 10, 17).

Cytopathology and histopathology

In cytopathology exfoliating cells of the cervix can be evaluated. Premalignant lesions of squamous cells are referred to as atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intra-epithelial lesions (HSIL). Glandular lesions are referred to as atypical glandular cells or atypical endocervical cells and adenocarcinoma in situ (AIS) (Bethesda system 2001) (18). In histopathology, premalignant squamous lesions are referred to as cervical intraepithelial neoplasia (CIN). Depending on the proportion of the epithelial layers showing dysplastic characteristics, squamous lesions are classified as mild (CIN I), moderate (CIN II), and severe dysplasia or carcinoma in situ (CIN III). The glandular premalignant lesions are classified as adenocarcinoma in situ (AIS). When the lesion breaks through the basal membrane and becomes invasive, it is referred to as squamous cell carcinoma (SCC) or adenocarcinoma (ADC).

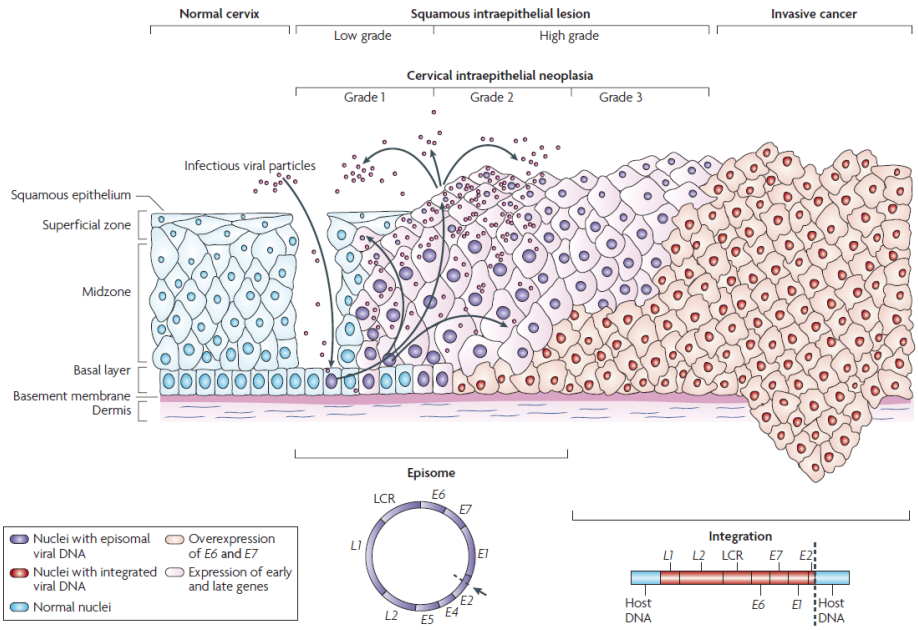


Figure 1 | **HPV-mediated progression to cervical cancer.** Basal cells in the cervical epithelium rest on the basement membrane, which is supported by the dermis. Human papillomavirus (HPV) is thought to access the basal cells through micro-abrasions in the cervical epithelium. Following infection, the early HPV genes *E1*, *E2*, *E4*, *E5*, *E6* and *E7* are expressed and the viral DNA replicates from episomal DNA (purple nuclei). In the upper layers of epithelium (the midzone and superficial zone) the viral genome is replicated further, and the late genes *L1* and *L2*, and *E4* are expressed. L1 and L2 encapsidate the viral genomes to form progeny virions in the nucleus. The shed virus can then initiate a new infection. Low-grade intraepithelial lesions support productive viral replication. An unknown number of high-risk HPV infections progress to high-grade cervical intraepithelial neoplasia (HGCIN). The progression of untreated lesions to microinvasive and invasive cancer is associated with the integration of the HPV genome into the host chromosomes (red nuclei), with associated loss or disruption of *E2*, and subsequent upregulation of *E6* and *E7* oncogene expression. LCR, long control region.

Adapted from Woodman et al (14)

HPV types

Over 150 HPV types have been identified. About 40 types are known to infect the mucosal epithelium of the human genital tract (17, 19).

HPV prevalence, age-specific prevalence, and type of distribution differ substantially between populations (20-23).

Almost all cervical cancer cases are caused by 15 types of HPV, i.e., 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59,73,68 and 82. Worldwide HPV 16 and HPV 18 are the most prevalent oncogenic HPV types and have shown to be responsible for respectively 54.6% and 15.8% of all cervical cancers (24-27). Combined HPV16/18 prevalence among invasive cervical cancer cases was slightly higher in Europe, North America and Australia (74–77%) than in Africa, Asia, and South/Central America (65–70%) yet data on HPV prevalence in invasive cervical cancer were particularly scarce from large regions of Africa and Central Asia. The next most common HPV types were the same in each continent, namely HPV31, 33, 35, 45, 52 and 58, although their importance differed by region (25).

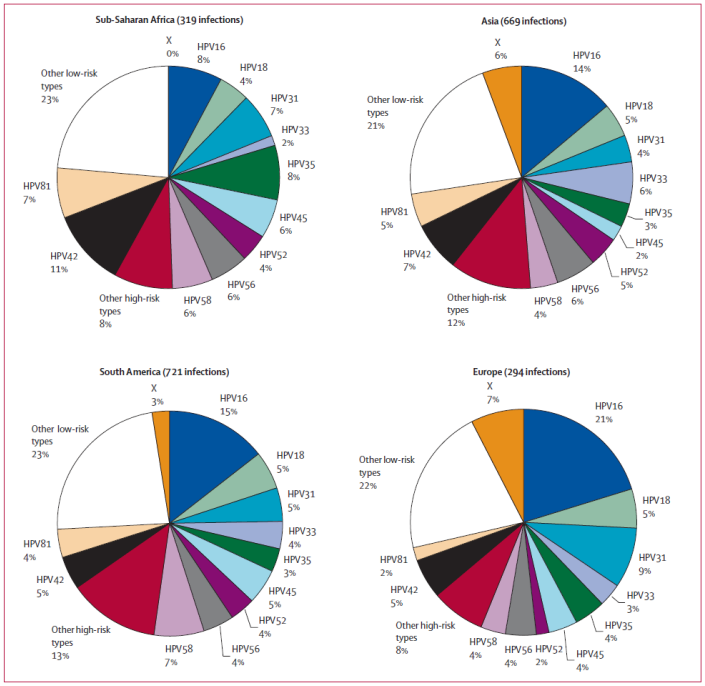


Figure 1: **HPV infections by type and region**

HPV types in cervical cytology normal women Adapted from Clifford et al (23)

Clades

The 40 HPV types that infect the epithelial mucosa of the genital tract can be classified into 15 different species or clades (α1-α15). The HPV types within these different clades are likely to have similar biological and medical properties (16, 28-32).

Two interesting clades are clade α-9 and clade α-7, both representing a group of HPV types with high oncogenic potential. The HPV types phylogenetically related and grouped as clade α-9 are HPV 16, 31, 33,35,52, and 58, the HPV types phylogenetically related and grouped as clade a-7 are HPV 18,39,45,59, and 68.

The two clades show differences in preference of site of infection, clade α-9 genotypes are more associated with squamous cell carcinomas (SCC) which develops at the ectocervix. The cladeα-7 genotypes are more commonly associated with adenocarcinomas (ADC) which develops from the mucus-producing glandular cells of the endocervix Although most HPV types in these clades induce both ADC and SCC, the difference in association may be caused by a greater tropism for infection and/or a better ability to neoplastically transform the glandular tissue or squamous cell tissue (31, 33-35).

4. Immunology

The human immune system consists of a non-specific innate immune system and an antigen-specific adaptive immune system. The non-specific innate immunity has no memory, but it cooperates with the antigen-specific adaptive immune system by antigen presentation. This adaptive immune system comprises B cells and T cells that generate antigen specific effector cells (B lymphocytes, antibodies) and memory cells (T-lymphocytes, cell-mediated).

The cell-mediated immune response plays an important role in protection in an HPV infection (13, 36). In a persistent HPV infection or in cervical cancer, the natural immune response against HPV proteins may have been compromised. Healthy subjects predominantly displayed a strong T-cell response (type 1 CD4+ T helper (Th1)) against early viral HPV antigens, whilst cervical cancer patients show either an impaired or an absent T-cell response (37).

Vaccinations

The central role of HPV infection in the causation of cervical neoplasia has led to efforts to produce prophylactic and therapeutic vaccinations.

Prophylactic vaccinations

The aim of prophylactic vaccinations is to prevent HPV infection and development of cervical neoplasia. The development of virus-like-particles (VLP) vaccines are based on recombinant L1 protein into non-infectious capsids that contain no genetic material. The vaccines are administered by intramuscular injection, inducing high antibody titers, more than 80-100-fold higher than after natural infection (38). The antibodies have to neutralize the virus before it infects the epithelium. Girls aged 9-14 should be vaccinated before they get sexually active and are possibly exposed to HPV.

The vaccine against HPV 16 and 18 (Cervarix GlaxoSmithKline Biologicals) and the vaccine against HPV 6, 11, 16, and 18 (Gardasil, Merck and Co.) have been tested in randomized placebo-controlled trials and shown to be safe, immunogenic, and highly efficacious. The trials have provided evidence of efficacy against infection with the types of HPV in the vaccines, against persistent infection and against the development of intra-epithelial lesions of the genital tract. In addition, Gardasil has shown to be highly efficacious against the development of genital warts caused by types 6 and 11(39-46). Since 2014 Gardasil 9 is approved by the US Food and Drug administration (FDA)(47). This vaccine is directed against HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 who are together responsible for almost 90% of the cervical cancers worldwide (48-50). Current guidelines recommend 2 vaccine doses to be fully protected, but there is evidence that show protection after a single dose (51, 52)

Therapeutic vaccinations

The aim of therapeutic vaccination is aiming at regression of existing HPV induced lesions. HPV induced high grade lesions (CIN III and cervical carcinoma) overexpress E6 and E7 oncoproteins. Therapeutic vaccinations are based on induction of specific immune response to these oncoproteins by aiming at induction or boosting HPV T-cell adaptive immunity. Various strategies to achieve effective therapeutic vaccines are being researched and progress is made. Encouraging results were shown in regression of high grade CIN lesions in correlation with a vaccine induced immune response but additional clinical studies are still necessary (53, 54). (55)

5. Cervical cancer prevention

As this disease can be detected and treated in early premalignant stages it is largely preventable, offering major opportunities to reduce mortality (56, 57). Efforts to increase awareness of disease is a very effective way to fight cancer (58). When women are aware about the disease and its symptoms, it's more likely they'll present in an early stage when the disease is still curable.

The ultimate solution is HPV vaccination to prevent primary infection and progressive disease (59, 60). As described Cervarix, Gardasil and Gardasil 9 are

available and have shown to be highly effective to reduce the risk of developing cervical cancer.

6. Secondary prevention, screening and treatment of precancerous lesions

The objective of cervical cancer screening is to prevent invasive cervical cancer by detecting and treating women with high grade cervical cancer precursor lesions (CIN 2/3). The effectiveness of screening is evaluated by the reduction in cervical cancer incidence and mortality observed in follow up. Organized programmes with systemic call, recall, follow up and surveillance systems have shown the greatest effect. The greatest reduction in cervical cancer incidence is reached when at least 80% of a population at risk is covered in the screening (57).

The screening must be performed when the prevalence of disease is highest, for premalignant cervical lesions the target age range is 30-49, generally 10 years before cancer develops. In low resource settings, because of the limited health care budgets, a small optimal age-group for cervical cancer screening to achieve the greatest public impact is 30-39 years old and screening even just twice in a lifetime is beneficial There must be sufficient health care infrastructure and human resources to provide the population with a well organised screening program. The screening test must be affordable and of good quality, meaning high sensitivity to identify disease and high specificity to minimalize the false negative results (61, 62).

In cervical cancer screening several approaches are used. First there are tests based on cytology; conventional pap smear, liquid-based cytology and dual staining to identify p16 and Ki67. Then there are tests making use of visual inspection; in combination with application of acetic acid to the cervix (VIA) or lugols iodine (VILI). Upcoming are automated visual evaluation of digital images and optoelectronic devices. Then there are also molecular tests based on nucleic acid amplification using HPV DNA or mRNA. Most commonly used are cytology, visual inspection, and HPV testing.

Conventional or liquid based cytology screening involves collection of exfoliating cells of the cervix, slide preparation, staining, reading and reporting. The mean sensitivity for conventional cytology (threshold ≥ ASCUS) to detect lesions CIN II has been described in a Cochrane review to range from 43-96% pooled 65.9% and from 39-85% pooled 70.3% for CIN 3. The specificity ranged from 86-98% pooled 96.3% for CIN II and 85-98% pooled 96.7% for CIN III.

For liquid-based cytology (threshold ≥ ASCUS) the sensitivity ranged from 52-94% pooled 75.5% for detection of CIN II and 52-98% pooled 76% for CIN III. For the specificity these ranged 73-97% pooled 91.9% for CIN II and 73-97% pooled 91.2% for CIN III (63).

Visual inspection methods include visual inspection with acetic acid (VIA), visual inspection with acetic acid using magnification (VIAM) and visual inspection with lugol's iodine (VILI). The availability of the test result directly after application of the solution is a major logistic advantage in providing diagnosis and treatment. VIA is the most used and most researched test. The accuracy of VIA has been studied extensively, the mean sensitivity and specificity, positive predictive value and negative predictive value are respectively 80% (range 79-82%), 92% (range 91-92%) 10% (9-10%) (64) and 99% (65). VIAM was thought to increase sensitivity but studies with VIAM did not improve test performance over VIA (66). Test results from VILI indicate that VILI is more sensitive than VIA but more research in to assess the effect of the screening stategy should be done (67, 68).

An alternative to VIA is screening with a real time optoelectronic device. These are handheld and use electrical and optical signals to classify cervical tissue in normal and abnormal by using a combination of biosensors including directly reflected light, backscattered light, and electrical decay curves. Transmitting light at specific frequencies through the cervical tissue examines the surface epithelial cells and identifies changes in the basal layer and stromal cells. Earlier studies described real time optoelectronic devices to be safe and effective in detecting premalignant lesions (69) and to improve the detection rate of CIN I and CIN II when it was used in combination with conventional cytology (70). Advantages of an optoelectronic device are that the result is directly available, it is objective, it is cheap and easy to use. As clinical research is limited, more research should be done comparing this device to other screening methods.

HPV testing is more sensitive than cytology in identifying cervical premalignant lesions. The most used tests are Hybrid capture -II and PCR tests, overall PCR tests are more sensitive to identify HPV infections. Using PCR tests sensitivity for detection of CIN II was found to range from 75-100% and from 88-100% for CIN III. For the specificities this was found to be respectively 85-97% and 79-94 (63). The specificity is lower in women younger than 30 where HPV infection usually is transient (71).

7. Treatment

Women with cytological low-grade lesions are generally advised to return for routine follow up smears, women with cytological high-grade lesions are further evaluated via colposcopy, biopsy and subsequent treatment of confirmed lesions. There are different methods of treatment; large loop excision of the transformation zone (LLETZ) and cryotherapy which can be performed in an outpatient setting. Laser conisation, knife conisation and laser ablation are usually performed under general anesthesia. The procedures performed in outpatient setting will be discussed below.

LLETZ or loop electrosurgical excision procedure (LEEP) utilizes a high-voltage, high-frequency alternating current that is passed through a thin electric wire loop

electrode to excise the abnormal area of the cervix. It provides a reliable tissue specimen for histological confirmation. Cure rates based on LLETZ are 90-95%, complications are mostly bleeding and infection (72, 73).

Cryotherapy can be used for the ablation of CIN. Cryotherapy involves freezing abnormal areas on the cervix, using compressed carbon dioxide (CO_2) or nitrous oxide (N_2O) as refrigerant. Cure rates based on histology of 95.0% for CIN I, 92.0% for CIN II and 86.0% for CIN III are achieved, and complications or adverse effects are rare. It is widely used and increases popularity in single visit approach (screen and treat) cervical cancer screening program mostly in low- and middle-income countries where cervical cancer is a real burden. It is suitable for the low resource settings as it is acceptable, affordable, safe and there is no need for electricity or anaesthesia. The possibility of direct treatment after diagnosing premalignant lesions, increases the effectiveness of the screening program as it decreases the loss of patients due to follow up (72, 74, 75).

TABLE 1
Two Outpatient Treatment Options for Precancer

	Cryotherapy (freezing)	LEEP (excision)
Effectiveness in eliminating precancerous tissue	86%-95%	90%-95%
Potential side effects	Watery discharge	Bleeding, infection
Anesthesia required	No	Yes
Tissue sample obtained	No	Yes
Power required	No	Yes
Cost	Relatively low	Relatively high

SOURCES: Adapted from Alliance for Cervical Cancer Prevention (ACCP), "Effectiveness, Safety, and Acceptability of Cryotherapy: A Systematic Literature Review" (2003); ACCP, "Treating Precancerous Cervical Lesions" (2004); and A. Bishop et al., "Cervical Cancer: Evolving Prevention Strategies for Developing Countries" (1995).

8. Indonesia

Indonesia, an archipelago in Southeast Asia, consists of more than 13,677 islands with a population of approximately 237 million of people, over 10 million people live in its capital Jakarta on the island of Java (Statistic Centre at Republic of Indonesia 2010). Gross National Product per Capita is 690.00 USD, the population consists of 49.86% females with a life expectancy of 69 years (76).

Cervical cancer in Indonesia

Cervical cancer concerns a major health problem in Indonesia, as it is the second leading cause of female cancer. The annual crude incidence rate is 27.0 per 100.000 women and estimated over a 36.600 new cases annually (77)

The governmental hospitals report cervical cancer to be up to 28% among all female cancer cases, representing 75% of all gynecological cancers. Approximately 70% is diagnosed in advanced stages (≥ FIGO stage IIB) (76, 78, 79).

There were no national screening programmes in Indonesia, until in 2007 the Ministry of Health started the Cervical and Breast Cancer Prevention (CECAP) project. This project was aiming for nationwide cervical cancer screening. Women aged 30-50 years old, were screened using visual inspection with acetic acid (VIA) and treatment with cryotherapy. In 2018, the cervical cancer screening coverage was 7.3% of the targeted population and available in 8 of the 34 provinces in Indonesia (80). The major issues remain the lack of awareness and participation in cervical cancer screening, especially for the women in low resource areas, with lower education and restricted access to health services (81).

According to a few studies the prevalent HPV types in Indonesia differ from the rest of the world as HPV 18 has a more predominant role in cervical cancer. According to Schellekens et al, HPV 18 was found as frequently as HPV 16 in cervical cancer (respectively 37.8% and 41.9%), and according to Bosch et al, HPV 18 was found even more frequently than HPV 16 (respectively 48.9% and 31.9%) (27, 82). More insight in prevalent HPV types in Indonesia is important for HPV vaccinations.

9. Outline of this thesis

In low- and middle-income countries, cervical cancer is the second most common cancer after breast cancer. Here, more than 85% of the worldwide new cervical cancer cases occur.

In developed countries, introduction of organized cytological screening with treatment led to tremendous drops in cervical cancer rates, as it allows detection and treatment of precancerous lesions and early-stage cervical cancer. Unfortunately, the health care infrastructure in low- and middle-income countries often prohibits successful implementation of organized screening due to lack of financial support, professional human resources, and laboratory services.

For many years research has been done to develop alternative screen and treatment approaches for low resource settings. Single visit approaches, where visual inspection with acetic acid (VIA) and cryotherapy are applied in the same visit, are feasible, highly efficient, and cost effective for these settings. Promising successes in decreasing cervical cancer incidence rates could be achieved using this method,

reducing the lifetime risk of cervical cancer by 25% when 35-year-old women are screened only once in their life.

The ultimate goal would be a world without cervical cancer, which could be obtained by a fully preventive and therapeutic vaccination available for all women and man in the world.

Knowledge of prevalent HPV types in a region has important implications for future vaccination strategies. Indonesia is a country with a high cervical cancer incidence and is known to have the world's highest prevalence of HPV 18 in cervical cancer. In **Chapter 2** we describe a hospital-based case control study performed in Jakarta, Indonesia, investigating the prevalent HPV types in the healthy hospital population.

In **Chapter 3**, we investigated age-specific prevalence of HPV types and possible risk factors of HPV positivity in a population-based sample of 2686 women, aged 15-70 years, in Jakarta, Tasikmalaya, and Bali, Indonesia.

In **Chapter 4** a population based single visit cervical cancer screening program is described, using visual inspection with acetic acid (VIA), histology and cryotherapy, in low resource areas in Jakarta and Tasikmalaya on the island of Java and on the island of Bali, Indonesia. The program was organized in collaboration with regional universities, regional hospitals, local health workers, the Indonesian cancer association and the national Indonesian family welfare organization. In mobile clinics selected areas were visited and the women were informed, screened and if needed immediately treated. The purpose was to evaluate the performance of the "See and treat"- method for cervical cancer screening in the Indonesian setting.

Of a group of women treated with cryotherapy in the See and treat program, samples for HPV testing were available before and half a year after cryotherapy. The persistence of HPV types after cryotherapy was investigated and described in **Chapter 5**. Special attention was there for types associated with glandular cell involvement as these mostly lie deeper within the cervix.

In **Chapter 6** we evaluated cervical screening with an optoelectronic device Truscreen; another alternative to cytology screening. In a teriary hospital setting we assessed the detection of CIN 2+ lesions by Truscreen in comparison to Liquid Based Cytology (LBC) and HPV DNA testing.

A summary and general discussion with future implications is given in **Chapter 7**, with a Dutch summary in **Chapter 8**.

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