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

Vet, J.N.

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

Jessica Vet

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

Jessica Vet

ISBN 9789464911602
Cover design: Female Cancer Foundation, www.femalecancerfoundation.org
Lay out, printing: www.proefschriftspecialist.nl
Financial support:
Financial support for the publication of this thesis was kindly provided by:
Female Cancer Foundation, www.femalecancerfoundation.org

Screening in low resource settings,

towards a world without cervical cancer

Proefschrift

ter verkrijging van

de graad van doctor aan de Universiteit Leiden,

op gezag van rector magnificus prof. dr. ir. H. Bijl,

volgens besluit van het college voor promoties

te verdedigen op woensdag 15 November 2023

klokke 11:15 uur

door

Jessica Naomi Immerentia Vet

geboren te Amsterdam

in 1976

Promotie commissie

Promotores:	Prof. Dr. G.J. Fleuren
	Prof. Dr. J.B.M.Z. Trimbos
Co-promotor:	Dr. M.A. de Boer
Promotie commissie:	Prof. Dr. J.M.M. van Lith
	Prof. Dr. N.F. Hacker
	Prof. Dr. M.J.E. Mourits
	Prof. Dr. V.T.H.B.M. Smit
	Dr. J.J. Beltman

"*Niet zonder hoofd, nooit zonder hart*"
Naar P.C. Hooft

Table of contents

Chapter 1

General introduction

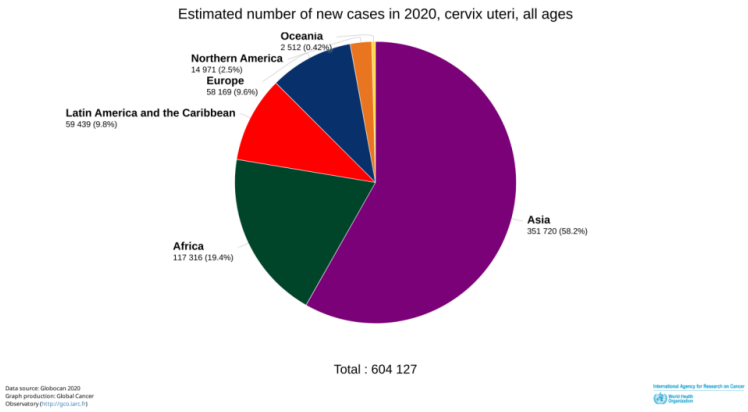
Chapter 1

General introduction

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).

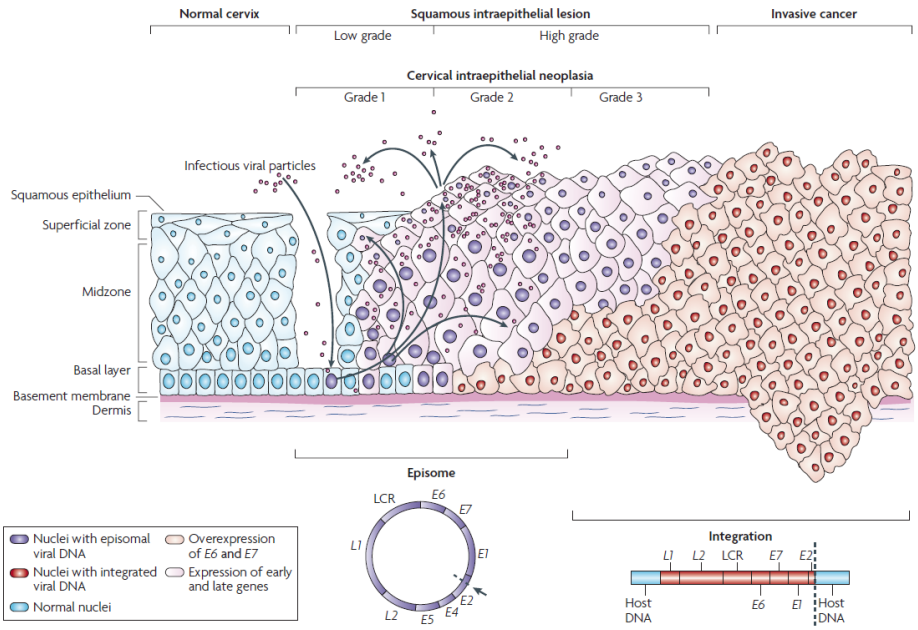


Figure 1 | **HPV-mediated progression to cervical cancer.** Basal cells in the cervical epithelium rest on the basement membrane, which is supported by the dermis. Human papillomavirus (HPV) is thought to access the basal cells through micro-abrasions in the cervical epithelium. Following infection, 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).

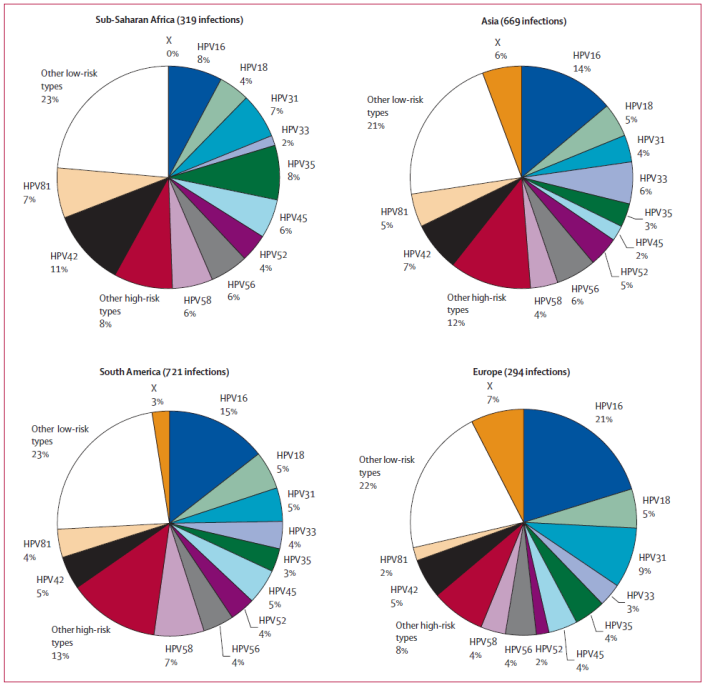


Figure 1: **HPV infections by type and region**

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**.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: a cancer journal for clinicians. 2021;71(3):209-49. Epub 2021/02/05.
2. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144(8):1941-53. Epub 2018/10/24.
3. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. The Journal of pathology. 1999;189(1):12-9. Epub 1999/08/19.
4. zur Hausen H. Human papillomaviruses in the pathogenesis of anogenital cancer. Virology. 1991;184(1):9-13. Epub 1991/09/01.
5. Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. Journal of clinical pathology. 2002;55(4):244-65. Epub 2002/03/29.
6. de Sanjose S, Brotons M, Pavon MA. The natural history of human papillomavirus infection. Best practice & research Clinical obstetrics & gynaecology. 2018;47:2-13. Epub 2017/10/02.
7. Burchell AN, Winer RL, de Sanjosé S, Franco EL. Chapter 6: Epidemiology and transmission dynamics of genital HPV infection. Vaccine. 2006;24 Suppl 3:S3/52-61. Epub 2006/09/05.
8. Smith JS, Melendy A, Rana RK, Pimenta JM. Age-specific prevalence of infection with human papillomavirus in females: a global review. The Journal of adolescent health : official publication of the Society for Adolescent Medicine. 2008;43(4 Suppl):S5-25, S.e1-41. Epub 2008/10/01.
9. Plummer M, Schiffman M, Castle PE, Maucort-Boulch D, Wheeler CM. A 2-year prospective study of human papillomavirus persistence among women with a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. The Journal of infectious diseases. 2007;195(11):1582-9. Epub 2007/05/02.
10. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet (London, England). 2007;370(9590):890-907. Epub 2007/09/11.
11. Crosbie EJ, Einstein MH, Franceschi S, Kitchener HC. Human papillomavirus and cervical cancer. Lancet (London, England). 2013;382(9895):889-99. Epub 2013/04/27.
12. Winer RL, Kiviat NB, Hughes JP, Adam DE, Lee SK, Kuypers JM, et al. Development and duration of human papillomavirus lesions, after initial infection. The Journal of infectious diseases. 2005;191(5):731-8. Epub 2005/02/03.
13. Schiffman M, Wentzensen N. Human papillomavirus infection and the multistage carcinogenesis of cervical cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2013;22(4):553-60. Epub 2013/04/04.
14. Woodman CB, Collins SI, Young LS. The natural history of cervical HPV infection: unresolved issues. Nature reviews Cancer. 2007;7(1):11-22. Epub 2006/12/23.

15. Castle PE, Einstein MH, Sahasrabuddhe VV. Cervical cancer prevention and control in women living with human immunodeficiency virus. CA: a cancer journal for clinicians. 2021;71(6):505-26. Epub 2021/09/10.
16. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. Virology. 2004;324(1):17-27. Epub 2004/06/09.
17. Doorbar J, Egawa N, Griffin H, Kranjec C, Murakami I. Human papillomavirus molecular biology and disease association. Reviews in medical virology. 2015;25 Suppl 1:2-23. Epub 2015/03/11.
18. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. Jama. 2002;287(16):2114-9. Epub 2002/04/23.
19. Bernard HU, Burk RD, Chen Z, van Doorslaer K, zur Hausen H, de Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology. 2010;401(1):70-9. Epub 2010/03/09.
20. Forman D, de Martel C, Lacey CJ, Soerjomataram I, Lortet-Tieulent J, Bruni L, et al. Global burden of human papillomavirus and related diseases. Vaccine. 2012;30 Suppl 5:F12-23. Epub 2012/12/05.
21. Bruni L, Diaz M, Castellsagué X, Ferrer E, Bosch FX, de Sanjosé S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. The Journal of infectious diseases. 2010;202(12):1789-99. Epub 2010/11/12.
22. Franceschi S, Herrero R, Clifford GM, Snijders PJ, Arslan A, Anh PT, et al. Variations in the age-specific curves of human papillomavirus prevalence in women worldwide. Int J Cancer. 2006;119(11):2677-84. Epub 2006/09/23.
23. Clifford GM, Gallus S, Herrero R, Munoz N, Snijders PJ, Vaccarella S, et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet (London, England). 2005;366(9490):991-8. Epub 2005/09/20.
24. de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. Int J Cancer. 2017;141(4):664-70. Epub 2017/04/04.
25. Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer. 2007;121(3):621-32. Epub 2007/04/04.
26. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. The Lancet Oncology. 2010;11(11):1048-56. Epub 2010/10/19.
27. Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. Journal of the National Cancer Institute. 1995;87(11):796-802. Epub 1995/06/07.
28. Bernard HU. The clinical importance of the nomenclature, evolution and taxonomy of human papillomaviruses. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2005;32 Suppl 1:S1-6. Epub 2005/03/09.
29. Burk RD, Chen Z, Van Doorslaer K. Human papillomaviruses: genetic basis of carcinogenicity. Public health genomics. 2009;12(5-6):281-90. Epub 2009/08/18.

30. Doorbar J. Molecular biology of human papillomavirus infection and cervical cancer. Clinical science (London, England : 1979). 2006;110(5):525-41. Epub 2006/04/07.
31. Clifford G, Franceschi S. Members of the human papillomavirus type 18 family (alpha-7 species) share a common association with adenocarcinoma of the cervix. Int J Cancer. 2008;122(7):1684-5. Epub 2007/12/07.
32. de Villiers EM. Cross-roads in the classification of papillomaviruses. Virology. 2013;445(1-2):2-10. Epub 2013/05/21.
33. Seoud M, Tjalma WA, Ronsse V. Cervical adenocarcinoma: moving towards better prevention. Vaccine. 2011;29(49):9148-58. Epub 2011/10/11.
34. Bulk S, Berkhof J, Bulkmans NW, Zielinski GD, Rozendaal L, van Kemenade FJ, et al. Preferential risk of HPV16 for squamous cell carcinoma and of HPV18 for adenocarcinoma of the cervix compared to women with normal cytology in The Netherlands. British journal of cancer. 2006;94(1):171-5. Epub 2006/01/13.
35. Li N, Franceschi S, Howell-Jones R, Snijders PJ, Clifford GM. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. Int J Cancer. 2011;128(4):927-35. Epub 2010/05/18.
36. Stanley M. Immunobiology of HPV and HPV vaccines. Gynecologic oncology. 2008;109(2 Suppl):S15-21. Epub 2008/06/14.
37. Einstein MH. Acquired immune response to oncogenic human papillomavirus associated with prophylactic cervical cancer vaccines. Cancer immunology, immunotherapy : CII. 2008;57(4):443-51. Epub 2007/12/25.
38. Villa LL, Ault KA, Giuliano AR, Costa RL, Petta CA, Andrade RP, et al. Immunologic responses following administration of a vaccine targeting human papillomavirus Types 6, 11, 16, and 18. Vaccine. 2006;24(27-28):5571-83. Epub 2006/06/07.
39. Arbyn M, Xu L, Simoens C, Martin-Hirsch PP. Prophylactic vaccination against human papillomaviruses to prevent cervical cancer and its precursors. The Cochrane database of systematic reviews. 2018;5(5):Cd009069. Epub 2018/05/10.
40. Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. Lancet (London, England). 2004;364(9447):1757-65. Epub 2004/11/16.
41. Villa LL, Costa RL, Petta CA, Andrade RP, Ault KA, Giuliano AR, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. The Lancet Oncology. 2005;6(5):271-8. Epub 2005/05/03.
42. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. The New England journal of medicine. 2007;356(19):1915-27. Epub 2007/05/15.
43. Roteli-Martins CM, Naud P, De Borba P, Teixeira JC, De Carvalho NS, Zahaf T, et al. Sustained immunogenicity and efficacy of the HPV-16/18 AS04-adjuvanted vaccine: up to 8.4 years of follow-up. Human vaccines & immunotherapeutics. 2012;8(3):390-7. Epub 2012/02/14.
44. Castle PE, Maza M. Prophylactic HPV vaccination: past, present, and future. Epidemiology and infection. 2016;144(3):449-68. Epub 2015/10/03.
45. Hariri S, Bennett NM, Niccolai LM, Schafer S, Park IU, Bloch KC, et al. Reduction in HPV 16/18-associated high grade cervical lesions following HPV vaccine introduction in the United States - 2008-2012. Vaccine. 2015;33(13):1608-13. Epub 2015/02/15.

46. Donovan B, Franklin N, Guy R, Grulich AE, Regan DG, Ali H, et al. Quadrivalent human papillomavirus vaccination and trends in genital warts in Australia: analysis of national sentinel surveillance data. The Lancet Infectious diseases. 2011;11(1):39-44. Epub 2010/11/12.
47. Printz C. FDA approves Gardasil 9 for more types of HPV. Cancer. 2015;121(8):1156-7. Epub 2015/04/10.
48. Herrero R, Gonzalez P, Markowitz LE. Present status of human papillomavirus vaccine development and implementation. The Lancet Oncology. 2015;16(5):e206-16. Epub 2015/05/07.
49. Human papillomavirus vaccines: WHO position paper, May 2017. Releve epidemiologique hebdomadaire. 2017;92(19):241-68. Epub 2017/05/23. Vaccins contre les papillomavirus humains: note de synthese de l'OMS, mai 2017.
50. Harper DM, DeMars LR. HPV vaccines - A review of the first decade. Gynecologic oncology. 2017;146(1):196-204. Epub 2017/04/27.
51. Brotherton JM, Budd A, Rompotis C, Bartlett N, Malloy MJ, Andersen RL, et al. Is one dose of human papillomavirus vaccine as effective as three?: A national cohort analysis. Papillomavirus research (Amsterdam, Netherlands). 2019;8:100177. Epub 2019/07/19.
52. Stanley M, Dull P. HPV single-dose vaccination: Impact potential, evidence base and further evaluation. Vaccine. 2018;36(32 Pt A):4759-60. Epub 2018/05/15.
53. Yang A, Farmer E, Wu TC, Hung CF. Perspectives for therapeutic HPV vaccine development. Journal of biomedical science. 2016;23(1):75. Epub 2016/11/05.
54. Hancock G, Hellner K, Dorrell L. Therapeutic HPV vaccines. Best practice & research Clinical obstetrics & gynaecology. 2018;47:59-72. Epub 2017/11/08.
55. Trimble CL, Morrow MP, Kraynyak KA, Shen X, Dallas M, Yan J, et al. Safety, efficacy, and immunogenicity of VGX-3100, a therapeutic synthetic DNA vaccine targeting human papillomavirus 16 and 18 E6 and E7 proteins for cervical intraepithelial neoplasia 2/3: a randomised, double-blind, placebo-controlled phase 2b trial. Lancet (London, England). 2015;386(10008):2078-88. Epub 2015/09/21.
56. Denny L. Cervical cancer: prevention and treatment. Discovery medicine. 2012;14(75):125-31. Epub 2012/09/01.
57. Sankaranarayanan R, Budukh AM, Rajkumar R. Effective screening programmes for cervical cancer in low- and middle-income developing countries. Bulletin of the World Health Organization. 2001;79(10):954-62. Epub 2001/11/06.
58. Vineis P, Wild CP. Global cancer patterns: causes and prevention. Lancet (London, England). 2014;383(9916):549-57. Epub 2013/12/20.
59. Pollack AE, Balkin MS, Denny L. Cervical cancer: a call for political will. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2006;94(3):333-42. Epub 2006/07/22.
60. Vaccarella S, Lortet-Tieulent J, Plummer M, Franceschi S, Bray F. Worldwide trends in cervical cancer incidence: impact of screening against changes in disease risk factors. European journal of cancer (Oxford, England : 1990). 2013;49(15):3262-73. Epub 2013/06/12.
61. Sherris J, Wittet S, Kleine A, Sellors J, Luciani S, Sankaranarayanan R, et al. Evidence-based, alternative cervical cancer screening approaches in low-resource settings. International perspectives on sexual and reproductive health. 2009;35(3):147-54. Epub 2009/10/07.
62. WHO Guidelines Approved by the Guidelines Review Committee. WHO guideline for screening and treatment of cervical pre-cancer lesions for cervical cancer prevention. Geneva: World Health Organization

63. Koliopoulos G, Nyaga VN, Santesso N, Bryant A, Martin-Hirsch PP, Mustafa RA, et al. Cytology versus HPV testing for cervical cancer screening in the general population. The Cochrane database of systematic reviews. 2017;8(8):Cd008587. Epub 2017/08/11.
64. Sauvaget C, Fayette JM, Muwonge R, Wesley R, Sankaranarayanan R. Accuracy of visual inspection with acetic acid for cervical cancer screening. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2011;113(1):14-24. Epub 2011/01/25.
65. Sankaranarayanan R, Nene BM, Shastri SS, Jayant K, Muwonge R, Budukh AM, et al. HPV screening for cervical cancer in rural India. The New England journal of medicine. 2009;360(14):1385-94. Epub 2009/04/03.
66. Sankaranarayanan R, Rajkumar R, Theresa R, Esmy PO, Mahe C, Bagyalakshmi KR, et al. Initial results from a randomized trial of cervical visual screening in rural south India. Int J Cancer. 2004;109(3):461-7. Epub 2004/02/13.
67. Sankaranarayanan R, Wesley R, Thara S, Dhakad N, Chandralekha B, Sebastian P, et al. Test characteristics of visual inspection with 4% acetic acid (VIA) and Lugol's iodine (VILI) in cervical cancer screening in Kerala, India. Int J Cancer. 2003;106(3):404-8. Epub 2003/07/08.
68. Fokom-Domgue J, Combescure C, Fokom-Defo V, Tebeu PM, Vassilakos P, Kengne AP, et al. Performance of alternative strategies for primary cervical cancer screening in sub-Saharan Africa: systematic review and meta-analysis of diagnostic test accuracy studies. BMJ (Clinical research ed). 2015;351:h3084. Epub 2015/07/05.
69. Pruski D, Kedzia W, Przybylski M, Jozefiak A, Kedzia H, Spaczynski M. [Assesment of real optoelectronic method in the detection of cervical intraepithelial neoplasia]. Ginekologia polska. 2008;79(5):342-6. Epub 2008/07/16. Ocena przydatnosci metody optoelektronicznej w wykrywaniu srodnablonkowej neoplazji szyjki macicy.
70. Singer A, Coppleson M, Canfell K, Skladnev V, Mackellar G, Pisal N, et al. A real time optoelectronic device as an adjunct to the Pap smear for cervical screening: a multicenter evaluation. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2003;13(6):804-11. Epub 2003/12/17.
71. Arbyn M, Roelens J, Simoens C, Buntinx F, Paraskevaidis E, Martin-Hirsch PP, et al. Human papillomavirus testing versus repeat cytology for triage of minor cytological cervical lesions. The Cochrane database of systematic reviews. 2013(3):Cd008054. Epub 2013/04/02.
72. Martin-Hirsch PP, Paraskevaidis E, Bryant A, Dickinson HO. Surgery for cervical intraepithelial neoplasia. The Cochrane database of systematic reviews. 2013(12):Cd001318. Epub 2013/12/05.
73. Alliance for Cervical Cancer PreventionACCP. Effectiveness s, and acceptability of cryotherapy. A systematic literature review. Cervical cancer prevention issues in depth, no 10 (2003).
74. Sauvaget C, Muwonge R, Sankaranarayanan R. Meta-analysis of the effectiveness of cryotherapy in the treatment of cervical intraepithelial neoplasia. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2013;120(3):218-23. Epub 2012/12/26.
75. Santesso N, Schunemann H, Blumenthal P, De Vuyst H, Gage J, Garcia F, et al. World Health Organization Guidelines: Use of cryotherapy for cervical intraepithelial neoplasia. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2012;118(2):97-102. Epub 2012/06/26.
76. Aziz MF. Gynecological cancer in Indonesia. Journal of gynecologic oncology. 2009;20(1):8-10. Epub 2009/05/28.

77. (ES). IIIC. Human papillomavirus and related diseases in Indonesia. Summary report 17 June 2019. . Barcelona: ICO/IARC HPV Information Centre.
78. Tjindarbumi D, Mangunkusumo R. Cancer in Indonesia, present and future. Japanese journal of clinical oncology. 2002;32 Suppl:S17-21. Epub 2002/04/18.
79. Sutnick AI, Gunawan S. Cancer in Indonesia. Jama. 1982;247(22):3087-8. Epub 1982/06/11.
80. Aoki ES, Yin R, Li K, Bhatla N, Singhal S, Ocviyanti D, et al. National screening programs for cervical cancer in Asian countries. Journal of gynecologic oncology. 2020;31(3):e55. Epub 2020/04/09.
81. Anwar SL, Tampubolon G, Van Hemelrijck M, Hutajulu SH, Watkins J, Wulaningsih W. Determinants of cancer screening awareness and participation among Indonesian women. BMC cancer. 2018;18(1):208. Epub 2018/03/07.
82. Schellekens MC, Dijkman A, Aziz MF, Siregar B, Cornain S, Kolkman-Uljee S, et al. Prevalence of single and multiple HPV types in cervical carcinomas in Jakarta, Indonesia. Gynecologic oncology. 2004;93(1):49-53. Epub 2004/03/30.

Chapter 2

Human papillomavirus type 18 and other risk factorsfor cervical cancer in Jakarta, Indonesia

De Boer MA, Vet JN, Aziz MF, Cornain S, Purwoto G, van den Akker BE, Dijkman A, Peters AA, Fleuren GJ.

Adapted from Int J Gynecol Cancer. 2006 Sep-Oct;16(5):1809-14.

Abstract

Infection with human papillomavirus (HPV) has now been established as a necessary cause of cervical cancer. Indonesia is a country with a high cervical cancer incidence and with the world's highest prevalence of HPV 18 in cervical cancer. No information exists about the prevalence of HPV 18 or other HPV types in the Indonesian population. We conducted a hospital-based case-control study in Jakarta, Indonesia. A total of 74 cervical carcinoma cases and 209 control women, recruited from the gynecological outpatient clinic of the same hospital, were included. All women were HPV typed by the line probe assay, and interviews were obtained regarding possible risk factors for cervical cancer. HPV was detected in 95.9% of the cases and in 25.4% of the controls. In the control group, 13.4% was infected with a high-risk HPV type. HPV 16 was detected in 35% of the case group and in 1.9% of the control group and HPV 18 was identified in 28% of the case group and in 2.4% of the control group, suggesting that the oncogenic potentials of HPV 16 and HPV 18 in Indonesia are similar. In addition to HPV infection, young age at first intercourse, having a history of more than one sexual partner, and high parity were significant risk factors for cervical cancer. Within the control group, we did not identify determinants of HPV infection. We hypothesize that the high prevalence of HPV 18 in cervical cancer in Indonesia is caused by the high prevalence of HPV 18 in the Indonesian population.

Introduction

Cervical cancer is the second most common cancer in females worldwide. The incidence is highest in developing countries, largely as a result of lack of screening programs and poor access to medical care. The prevalence of human papillomavirus (HPV) and the distribution of its types probably plays an important role as well (1–3). Little is known about viral and epidemiologic factors in Indonesia, a country with a high cervical cancer incidence (4).

Infection with HPV has now been established as a necessary but not sufficient cause of cervical cancer (5). More than 80 HPV types have been identified and about 40 types can infect the genital tract. HPV types can be divided into low-risk and high-risk types according to their ability to induce cancer. Worldwide, HPV 16 is the most common high-risk type, present in 50%, followed by HPV 18, present in 14% of cervical cancers (6). Indonesia was described to have the highest prevalence of HPV 18 in cervical cancer in the world (6). The prevalence of HPV 18 in the Indonesian population is not known yet.

Apart from infection with HPV, several additional risk factors for cervical cancer have been studied. High parity, high number of sexual partners, smoking, young age at first intercourse, limited education, history of sexual transmitted infections, and low socioeconomic status have been proposed as additional risk factors (7–11). Yet, the importance of these factors seems to differ between populations and has never been studied in Indonesia. In this hospital-based case–control study, we assessed the risk associated with individual HPV types and possible additional risk factors in the etiology of cervical cancer in Indonesia.

Material and methods

Study population and specimen collection

The present study was a hospital-based case–control study. The case group consisted of patients diagnosed with invasive cervical cancer and was previously described by Schellekens *et al.* (4). A group of 104 first attendants with clinically a strong suspicion of cervical cancer was formed in the period October 2001 to March 2002 in the outpatient clinic of the National General Hospital "Dr Cipto Manungkusumo" in Jakarta, Indonesia. An oncologic gynecologist performed pelvic examination, staged according to FIGO classification, and biopsy specimens of the cervical lesion were taken and subsequently formalin-fixed and paraffin-embedded. Sixteen samples were not available for further analysis. The remaining 88 biopsy samples were histopathologically classified. In five specimens, the tissue was not distinctive enough, and in nine cases, we found cervical intraepithelial neoplasia. These 14 cases were therefore excluded from further analysis.

The control group consisted of 209 women without cervical cancer who were identified among patients attending the gynecological outpatient clinic of the same hospital for nonmalignant disease in the period December 2003 to January 2004.

Exclusion criteria included: diagnosis or suspicion of anogenital tract cancers, a history of hysterectomy or conization, current pregnancy, and mental incompetence. Accordingly, after informed consent was given, in a group of 213 women cervical scrapes of the endo and ectocervix were collected by sampling the cervix with an endocervical brush and a wooden Ayre spatula for the preparation of two Pap smears. The first smears were shipped to Leiden for DNA isolation and HPV typing. To prevent contamination, this smear was packed in a separate box for each patient and did not go through any staining procedures. The second smear was stained for cytologic classification. Trained technicians performed cytologic diagnosis according to the Bethesda classification. Their diagnoses, which were not used as a criterion for exclusion, were normal (*n*= 202), atypical cells of undetermined significance (ASCUS) (*n*= 1), atypical glandular cells of undetermined significance (AGUS) (*n*= 3), atypical glandular cells (*n*= 1), low-grade intraepithelial lesion (LSIL) (*n*= 1), high-grade intraepithelial lesion (HSIL) (*n*= 2) and one suspicious of adenocarcinoma. Two noninformative smears were excluded from further analysis.

Data collection

Personal interviews were obtained using standardized questionnaires that included information on ethnic background, socioeconomic status, smoking habits, reproductive history, use of contraceptives, and sexual behavior. Two female doctors administered the questionnaires in the hospital.

DNA isolation

DNA was isolated from the cervical tumor tissue as previously described (4). The isolation of DNA from cervical smears was performed as previously described by Jacobs *et al.* (12) using the commercially available High Pure PCR Template Preparation Kit (Boehringer-Mannheim, Indianapolis, IN). This method was described to provide a reliable means to extract DNA from archival smears with a minimal susceptibility to contamination(12). To test the quality of the isolated DNA, we performed a polymerase chain reaction on the human genomic β-globin gene. Two samples in the control group tested negative and were therefore excluded. All samples in the case group tested positive for β-globin. The final groups consisted of 74 case subjects (45 squamous cell carcinomas and 29 adenocarcinomas/adenosquamous carcinomas) and 209 control subjects. Characteristics of the patient and control group are depicted in Table 1.

HPV typing

HPV DNA detection and genotyping were performed as previously described (4). Shortly, DNA was amplified with the SPF10 primer, and the presence of HPV amplimers was tested on an agarose gel. Samples that tested negative were retested in a 1:10 dilution. HPV genotyping of positive products was performed by the INNO-line probe assay prototype research genotyping assay (Innogenetics, Gent, Belgium),

a highly sensitive hybridization assay that can simultaneously detect 25 HPV types (13).

Statistical analysis

Statistical analysis was performed using the SPSS 10.0.7 software package. To estimate the risk of cervical cancer associated with individual HPV types or lifestyle factors, we calculated odds ratios (ORs) and 95% confidence intervals (CIs) by using unconditional logistic regression. The Pearson Chi-square test for independence was calculated to detect statistically significant differences in the proportion of women who had a risk factor among the HPV positives versus HPV negatives. Differences were considered to be statistically significant when *P* values were below 0.05.

Table 1. Patient characteristics of case and control group

	Cervical cancer, $N = 74$	Normal controls, $N = 209$
Age, mean (years)	46.5	45.0
Ethnical group (%)		
Java	23	38
Sunda	31	23
Betawi	18	12
Sumatra	14	19
Sulawesi	5	2
Chinese	3	4
Other	4	2
Unknown	3	0

Results

HPV prevalence and distribution

The mean age for patients in the case group was 46.5 years and 45.0 years for women in the control group. Patients were distributed similarly by ethnicity. In the patient group, a total of 71/74 (95.9%) was proved to be HPV positive, and in the control group, 53/209 (25.4%) samples proved HPV positive. Of these control women, 13.4% were infected with a high-risk type, 7.7% with low-risk types, and 4.3% by an uncharacterized type (HPV X). We detected 11 different HPV types in the case group and 17 different types in the control group. The distribution of HPV types in the case group and control group is depicted in Table 2.

The most common HPV types in the case group were HPV 16 and HPV 18 in almost equal percentages, followed by HPV 52 and HPV 45. In the control group, the most common types were HPV 51, 74, 18, 16, 44, and 54, in descending order. The overall low prevalence of high-risk types in the control group resulted in an OR for cervical cancer for infections with a high-risk HPV type of 153.0. (95% CI, 45–519). The OR (with 95% CI) for HPV 16 was 26.1 (8.7–78.5) and 16.2 (5.8–44.9) for HPV 18. The distribution of HPV 16 and HPV 18 were not statistically different (Fisher's exact test $P = 0.72$).

Nine samples from the control group were positive by the SPF-10 primer set but could not be assigned to any of the known HPV types by line probe assay, and therefore referred to by HPV X. These samples were retested by the My09/11 primers. Three samples tested positive and through sequencing they were identified as HPV 62 in two cases and HPV 83 in one case. Multiple HPV types were present in 14% of the case group and in 2.4% of the control group. In the control group, the HPV 18-positive infections were all single infections, and one of the HPV 16-positive samples was a multiple infection with HPV 43.

Table 2. Distribution of HPV types in cancer patients versus controls. The classification in high- and low-risk types was performed according to Munoz *et al.*[19]

	Case subjects, *n* (%)	Control subjects, *n* (%)	OR (95% CI)
HPV negative	3 (4.1)	156 (68.4)	
Positive, any HPV type	71 (95.9)	53 (25.4)	69.5 (21–229.9)
Positive, any high-risk type[a]	71 (95.9)	28 (13.4)	153.0 (45.0–519.2)
HPV 16	25 (35.0)	4 (1.9)	26.1 (8.7–78.5)
HPV 18	21 (28.0)	5 (2.4)	16.2 (5.8–44.9)
HPV 52	6 (8.0)	1 (0.5)	18.4 (2.1–155.0)
HPV 45	4 (5.0)	2 (1.0)	5.9 (1.1–33.0)
HPV 51	0 (0.0)	6 (2.9)	0
HPV 74 (low risk)	0 (0.0)	5 (2.4)	0
Other high-risk types	5 (3.0)	3 (1.4)	5.0 (1.2–21.5)
Probable high-risk[b]	0 (0.0)	2 (0.5)	0
Multiple infections	10 (14.0)	5 (2.4)	6.4 (2.1–19.3)
HPV X	0 (0.0)	9 (4.3)	0
Other low-risk types	0	11 (5.3)	0

[a]Also including probable high-risk types and multiple infections that include high-risk types.
[b]Both HPV 53.

Other risk factors

In univariate regression analysis reporting young age at first intercourse, more than one sexual partner, and high parity were associated with risk of cervical cancer (Table 3). Smoking was more frequent in the case group, yet was not a significant risk factor.

Table 3. ORs for cervical cancer and corresponding 95% CI by sexual and reproductive risk factors and smoking

	Case subjects, $N = 66^a$, (%)	Control subjects, $N = 209$, (%)	OR (95% CI)	P value
Age at first intercourse (years)				
≤19	27 (56.1)	85 (40.7)	1.0 (referent)	0.012
≥20	29 (43.9)	124 (59.3)	0.48 (0.28–0.85)	
Number of sexual partners				
=1	40 (60.6)	188 (90.0)	1.0 (referent)	<0.0001
>1	26 (39.4)	21 (10.0)	5.83 (2.98–11.36)	
Parity				
0–3	27 (40.9)	136 (65.1)	1.0 (referent)	<0.001
>3	39 (59.1)	73 (34.9)	2.7 (1.55–4.72)	
Smoking status				
Never	47 (75.8)	175 (83.7)	1.0 (referent)	0.16
Ever	15 (24.2)	34 (16.3)	1.64 (0.81–3.31)	
Unknown	4			

[a]Eight cases from the case group were excluded because not all data were available.

Determinants of HPV infection

Among control subjects, HPV positivity was not associated with age, with 25% positive among women under 35 years, 25% in women aged 35–54 years, and 22% HPV positive in women of 55 years and older. Trends for HPV positivity were observed with decreasing parity (P= 0.08) and with older age at first intercourse (P= 0.08). We did not find an association of HPV prevalence with the number of sexual partners, the use of hormonal contraceptives, religion, or smoking. Determinants of HPV infection within the control group are presented in Table 4.

Table 4. Determinants of HPV infection within the control group

	HPV positive[a], $N = 53$	HPV negative, $N = 156$	P value[a]
Age (years)			0.79
<35	10 (18.9)	29 (18.6)	
35–44	19 (35.8)	45 (28.8)	
45–54	18 (34.0)	62 (39.7)	
≥55	6 (11.3)	20 (12.8)	
Parity			0.08
0–1	14 (26.4)	38 (24.4)	
2–3	27 (50.9)	57 (36.5)	
≥4	12 (22.6)	61 (39.1)	
Age at first intercourse (years)			0.08
<16	7 (13.2)	24 (15.4)	
17–19	8 (15.1)	46 (29.5)	
>20	38 (71.7)	86 (55.1)	
Sexual partners			0.48
=1	49 (92.5)	139 (89.1)	
>1	4 (7.5)	17 (10.9)	
Oral contraceptives			0.4
Never	29 (54.7)	75 (48.1)	
Ever	24 (45.3)	81 (51.9)	
Religion			0.82
Islam	41 (25.0)	123 (75.0)	
Other	12 (26.7)	33 (73.3)	
Smoking			0.79
Never	45 (84.9)	130 (83.3)	
Ever	8 (15.1)	26 (16.7)	

[a]HPV positive includes high-risk types, probable high-risk types, low-risk types, and HPV X.

Discussion

In this case–control study, we studied the prevalence of HPV 18 in a hospital-based population in Indonesia, the only country in the world described to have a similar or higher prevalence of HPV 18 compared to HPV 16 in cervical cancer patients. We found that the high prevalence of HPV 18 in cervical cancer patients is related to the high prevalence of HPV 18 in our control group.

The prevalence of HPV DNA in cervical smears is reported to be closely correlated with cervical cancer incidence rates (2). The prevalence of HPV among control women in our study 21.5% was comparable to the prevalence in high incidence countries like India 27.7% (8), Nigeria 26.3% (14), Chile 14.0% (15), and Colombia 14.8% (16). Consequently, the high HPV prevalence in Indonesia is in agreement with the high estimated incidence rate of cervical cancer in Indonesia of 25–40 per 100,000 women per year (4). Yet, our results may be biased because our control group was a selected group, consisting of women attending a gynecological outpatient clinic, both symptomatic and asymptomatic, and therefore may not be generalized to the general population.

The total number of multiple infections found was 2.4%, which is high compared with 1.2% as described in a worldwide study by Munoz (5). Yet, in areas with a high overall prevalence of HPV in the normal population, the percentage of multiple infections in the same group seems to be higher as demonstrated by Pham *et al.*(17).

Comparing the HPV prevalence in the case and the control group, we calculated an OR for HPV infection of 69.5 that is in agreement with risk estimates of 50–100 as reported in literature (18). The relative risk for infection with high-risk HPV types we found was 153.0. The distribution of HPV types in cervical cancer in this study was largely similar to that in normal smears. HPV 16 was observed in 35% of the cervical cancers, and HPV 18 was observed in 28%, whereas the prevalences in the control group were 1.9% and 2.4%, respectively. The ORs of HPV 16 and HPV 18 for progression to cervical cancer were within comparable ranges (HPV 16: OR, 26.1 (95% CI 8.7–78.5; HPV 18: OR, 16.2 (95% CI 5.8–44.9). Similar risks of progression to cervical cancer for HPV 16 and HPV 18 were observed in a worldwide study by Munoz *et al.*(19) as well. We conclude that, although the number of HPV 18–positive carcinomas is much larger in Indonesia compared to the rest of the world, the risk estimates for infection for progression to cervical cancer do not seem to be different and are similar to the risk of infection with HPV 16. Because the number of HPV 16– and HPV 18–positive samples in the control group is small, we cannot totally exclude other possible explanations for the high rate of HPV 18 in cervical cancer in Indonesia, like the prevalence of more oncogenic HPV variants and physiologic or immunologic causes. More research is needed, including also the distribution in precancerous disease, to draw definite conclusions.

HPV 51 had a high prevalence in our control group (2.9%) and was not identified in our case group, so behaves like a low-risk type in our group, yet was previously described as a high-risk type (19). In describing the prevalence of individual HPV

types, we recognize that the numbers in the control group are small, so risk calculations need to be addressed with caution. Also, the control group has not been collected in the same period as the case group.

In addition to HPV infection, first intercourse at the age of 19 or younger, having a history of more than one sexual partner, and high parity were significant risk factors for cervical cancer in Indonesia. Our numbers were too small to draw definite conclusions about additional risk factors; yet, results are concordant with world literature (7–10). By conducting a hospital-based case–control study, we tried to make case and control groups as comparable as possible. As shown in Table 1, the two groups are largely comparable as far as age and ethnic background are concerned. Still, patients who present at this government hospital with cervical cancer might be different compared to the women who visit the hospital with other health problems. Within the control group, we did not find an association between age and HPV positivity, probably because only a small number of women younger than 35 years were included. Muslim women have been described to have a low prevalence of cervical cancer and of HPV infection (20). Male circumcision is thought to provide a protective shelter to their female partners by getting fewer HPV infections (20). In our study, we did not identify differences between Muslim women and women with other religions, mostly Christians, neither was such a difference identified in a study conducted in India (20).

In conclusion, in Indonesia infection with a high-risk HPV type, young age at first intercourse, history of more than one sexual partner, and high parity were identified as risk factors for cervical cancer. The high prevalence of HPV in our control group is in agreement with the high estimated incidence rate of cervical cancer in Indonesia. The high prevalence of HPV 18 in cervical cancer patients is supposedly related to the high prevalence of HPV 18 in the general population and not due to a higher oncogenic potential of HPV 18 as compared to other HPV types.

References

1. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. Int J Cancer 1999;80:827–41.
2. Bosch FX, De Sanjose S. Chapter 1: Human papillomavirus and cervical cancer burden and assessment of causality. J Natl Cancer Inst Monogr 2003;31:3–13.
3. Berumen J, Ordonez RM, Lazcano E *et al.* Asian-American variants of human papillomavirus 16 and risk for cervical cancer: a case-control study. J Natl Cancer Inst 2001;93:1325–30.
4. Schellekens MC, Dijkman A, Aziz MF *et al.* Prevalence of single and multiple HPV types in cervical carcinomas in Jakarta, Indonesia. Gynecol Oncol 2004;93:49–53.
5. Munoz N. Human papillomavirus and cancer: the epidemiological evidence. J Clin Virol 2000;19:1–5.
6. Bosch FX, Manos MM, Munoz N *et al.* Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst 1995;87:796–802.
7. Chichareon S, Herrero R, Munoz N *et al.* Risk factors for cervical cancer in Thailand: a case-control study. J Natl Cancer Inst1998;90:50–7.
8. Franceschi S, Rajkumar T, Vaccarella S *et al.* Human papillomavirus and risk factors for cervical cancer in Chennai, India: a case-control study. Int J Cancer 2003;107:127–33.
9. Ngelangel C, Munoz N, Bosch FX *et al.* Causes of cervical cancer in the Philippines: a case-control study. J Natl Cancer Inst1998;90:43–9.
10. Hammouda D, Munoz N, Herrero R *et al.* Cervical carcinoma in Algiers, Algeria: human papillomavirus and lifestyle risk factors. Int J Cancer 2005;113:483–9.
11. Shields TS, Brinton LA, Burk RD *et al.* A case-control study of risk factors for invasive cervical cancer among U.S. women exposed to oncogenic types of human papillomavirus. Cancer Epidemiol Biomarkers Prev 2004;13:1574–82.
12. Jacobs MV, Zielinski D, Meijer CJ *et al.* A simplified and reliable HPV testing of archival Papanicolaou-stained cervical smears: application to cervical smears from cancer patients starting with cytologically normal smears. Br J Cancer 2000;82:1421–6.
13. Kleter B, Van Doorn LJ, Schrauwen L *et al.* Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. J Clin Microbiol 1999;37:2508–17.
14. Thomas JO, Herrero R, Omigbodun AA *et al.* Prevalence of papillomavirus infection in women in Ibadan, Nigeria: a population-based study. Br J Cancer 2004;90:638–45.
15. Ferreccio C, Prado RB, Luzoro AV *et al.* Population-based prevalence and age distribution of human papillomavirus among women in Santiago, Chile. Cancer Epidemiol Biomarkers Prev 2004;13:2271–6.
16. Molano M, Posso H, Weiderpass E *et al.* Prevalence and determinants of HPV infection among Colombian women with normal cytology. Br J Cancer 2002;87:324–33.
17. Pham TH, Nguyen TH, Herrero R *et al.* Human papillomavirus infection among women in South and North Vietnam. Int J Cancer2003;104:213–20.
18. Franceschi S. The IARC commitment to cancer prevention: the example of papillomavirus and cervical cancer. Recent Results Cancer Res 2005;166:277–97.
19. Munoz N, Bosch FX, De Sanjose S *et al.* Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518–27.
20. Duttagupta C, Sengupta S, Roy M *et al.* Are Muslim women less susceptible to oncogenic human papillomavirus infection? A study from rural eastern India. Int J Gynecol Cancer 2004;14:293–303.

Chapter 3

Prevalence of human papillomavirus in Indonesia: a population-based study in three regions

Vet JN, de Boer MA, van den Akker BE, Siregar B, Lisnawati, Budiningsih S, Tyasmorowati D, Moestikaningsih, Cornain S, Peters AA, Fleuren GJ.

Adapted from Br J Cancer. 2008 Jul 8;99(1):214-8.

Abstract

Cervical cancer is the most common cancer among women in the Indonesian population, yet little is known about the prevalence of human papillomavirus (HPV). 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.

The overall HPV prevalence was 11.4%, age-standardized to the world standard population 11.6%. The most prevalent types found were HPV 52, HPV 16, HPV 18, and HPV 39, respectively, 23.2, 18.0, 16.1, and 11.8% of the high-risk HPV types. In 20.7% of infections, multiple types were involved. Different age-specific prevalence patterns were seen: overall high in Jakarta, and in Tasikmalaya, and declining with age in Bali. The number of marriages was most associated with HPV positivity (OR 1.81 95% CI 1.31-2.51)).

Remarkably, in Indonesia HPV 16 and HPV 18 are equally common in the general population, as they are in cervical cancer. HPV 52 was the most prevalent type in the general population, suggesting that this type should be included when prophylactic HPV vaccination is introduced in Indonesia.

Introduction

Cervical cancer is the most common cancer in women in Indonesia, as in most developing countries (1,2). From hospital-based data, it accounts for 28.6% of female cancers in Indonesia (2).

Human papillomavirus (HPV) prevalence, age-specific prevalence, and type of distribution differ substantially between populations (3,4) and HPV 18 has a greater role in cervical cancer in Indonesia than in the rest of the world. HPV 18 was found as frequently as HPV 16 in cervical cancer (5), or even more frequently than HPV 16 (6). A small hospital-based case–control study conducted in Jakarta also found a high prevalence of HPV 18 in controls (7). In view of the lack of population-based relevant data, we report here the age-specific prevalence data for HPV among women in Jakarta and Tasikmalaya on the island of Java and among women on the island of Bali, and assess possible risk factors in the aetiology of cervical cancer

Material and methods

This population-based study was conducted as part of a screening project for cervical cancer in Jakarta and Tasikmalaya on the island of Java and different regions on the island of Bali in Indonesia between October 2004 and February 2006. Women were excluded if they were virgin, pregnant, had undergone a hysterectomy, or had previous cervical cancer. During our study it appeared that only married, divorced or widowed women participated in screening. Women who had never been married would strictly still be virgins because sexual intercourse before marriage is not allowed following Indonesia's cultural and religious rules.

To reach women, in each region a collaboration was set up with the Pembinaan Kesejahteraan Keluarga (PKK), the national Indonesian Family Welfare Organization. The smallest branches of this governmental women's movement organise activities at the village level. The attempt was made to invite all women aged 20–65 years in the selected villages and to screen at least 80% of these. Members of this local PKK invited participation by visiting women at home and informing them about risk factors, prevention, early detection, and treatment of premalignant cervical cancer lesions. Participants were individually counselled by public health nurses and their informed consent obtained; all were interviewed about socio-demographic, reproductive and cervical cancer risk factors.

A total of 20 834 women, aged 12–70, were screened: 6274 from Jakarta, 8007 from Tasikmalaya, and 6553 from Bali. For the HPV typing, a random age-stratified sample of the participants was taken for each region by categorising the women in 11 5-year age groups: $\leqslant$19, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49, 50–54, 55–59, 60–64, and $\geqslant$65 years. In each age group, 100 randomly selected samples were used for HPV

analysis. As the youngest and oldest groups were under-represented (<100 women), the total samples was less than 1100 per region. For Jakarta 915 were selected, for Tasikmalaya 975, and for Bali 950 samples.

Participants underwent a visual inspection of the cervix and a smear obtained using a wooden Ayre spatula for the ectocervix and a cytobrush for the endocervix. Slides were immediately fixed with ethanol and further processed for diagnosing by cytoscreeners. The exfoliated cells remaining on the spatula and brush were suspended in 25 ml of phosphate-buffered saline in a 50 ml Falcon tube. The tubes were centrifuged at 3000 g for 5 min. The supernatant was removed, and the cell pellet was re-suspended in 1 ml of phosphate-buffered saline and transferred to a 1.5 ml Eppendorf tube with a safety lock. All tubes were directly frozen and stored in a −20°C freezer and shipped on dry ice to the Department of Pathology, Leiden University Medical Center, the Netherlands.

Furthermore, all women underwent visual inspection with acetic acid and those women with acetowhite lesions and/or cytological abnormalities were treated with cryotherapy. In cases of suspected cervical cancer, the women were referred to the collaborating university hospitals.

To test the quality of DNA obtained from exfoliated cells, a polymerase chain reaction on the human genomic β-globin gene was performed. HPV DNA detection and genotyping were performed, amplified using the SPF10 primer and HPV amplimers tested on agarose gels (8).

The genotyping of positive products was performed using an INNO-line probe assay prototype research genotyping assay (Innogenetics, Ghent, Belgium which detected the following 25 types: HPV 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, and 74. High-risk types were identified using the HPV well known categories (9,10).

Statistical analysis

Statistical analysis was performed using SPSS software (SPSS, version 12, SPSS Inc., Chicago, IL, USA). For each region odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional multiple logistic regression adjusted for age were calculated to estimate the association between HPV infections and risk factors adjusted for age. To test for linear trend for odds ratios, a χ^2 linear test was calculated. Age standardization of rates for ages 15–70 was calculated using the world standard (11).

Results

Of the 2840 patients, 114 cellular samples were missing (26 Jakarta, 48 Tasikmalaya, 40 Bali). Of the remaining 2726, 40 samples were excluded because of a negative β-globin test (9 from Jakarta, 8 from Tasikmalaya, and 23 from Bali), of the final study group (2686 samples: 880 Jakarta, 919 Tasikmalaya, 887 from Bali). 91.2% had never been screened before, 80.7% from Jakarta, 97.4% Tasikmalaya and 95.6% Bali. Based on cytology, five of 880 women in Jakarta were diagnosed with cervical cancer, four of the 919 in Tasikmalaya and three of the 887 in Bali. Based on visual inspection with acetic acid, there were also two suspected cases in Tasikmalaya. In 13 of the 14 cases, the diagnoses were histologically confirmed and in the other the woman repeatedly refused follow up.

Overall, 305 samples (11.4%) were HPV positive (11.6% when world age-standardized), in Jakarta 122 samples (13.9% standardized 13.2%) were HPV positive, in Tasikmalaya 81 (8.8% standardized 9.0%), and in Bali 102 samples (11.5% standardized 12.1%). In total, in 211 samples (7.9%) high-risk HPV type was detected: 77 (8.9%) in Jakarta; 62 (6.7%) in Tasikmalaya and 72 (8.1%) in Bali. Forty-six samples (22 from Jakarta, 8 from Tasikmalaya, and 16 from Bali) were positive by the SPF10 primer set but not for HPV types represented in the line probe assay; these indicated as HPV X could not be categorised as cancer-associated or non-cancer-associated types.

Twenty-four different HPV types were detected, the commonest in descending order of prevalence being HPV 52, 16, and 18. This most differed by region, as shown in Table 1. Multiple HPV types were detected in 63 samples (2.3% overall, or 20.7% of all positive samples): in Jakarta in 22 (2.5 or 18.0%), in Tasikmalaya in 15 (1.6 or 18.5%), and in Bali in 26 (2.9 or 25.5%).

Table 1 Prevalence of type-specific cervical human papilloma infection types among 2686 women in Indonesia

	Jakarta (880)				Tasikmalaya (919)				Bali (887)			
HPV type[a]	**Single**	**Multiple**	**Total[b] (%)**	**HPV+[c] (%)**	**Single**	**Multiple**	**Total[b] (%)**	**HPV+[c] (%)**	**Single**	**Multiple**	**Total[b] (%)**	**HPV+[c] (%)**
Negative			758 (86.1)				838 (91.2)				785 (88.5)	
Positive	100	22	122 (13.9)		66	15	81 (8.8)		76	26	102 (11.5)	
High risk	56	22	78 (8.9)	63.9	47	15	62 (6.7)	76.5	52	25	77 (8.9)	75.4
Low risk	22	—	22 (2.5)	18.0	11	—	11 (1.2)	13.6	8	1	9 (1.0)	8.8
X	22	—	22 (2.5)	18.0	8	—	8 (0.9)	9.9	16	—	16 (1.8)	15.7
High risk												
16	10	3	13 (1.5)	10.7	5	5	10 (1.1)	12.3	8	7	15 (1.7)	15.0
18	5	3	8 (0.9)	6.6	10	4	14 (1.5)	17.3	7	5	12 (1.4)	12.0
31	—	1	1 (0.1)	0.8	1	—	1 (0.1)	1.2	2	1	3 (0.3)	3.0
33	1	1	2 (0.2)	1.6	—	1	1 (0.1)	1.2	3	3	6 (0.7)	5.9
35	3	—	3 (0.3)	2.5	2	1	3 (0.3)	3.7	2	—	2 (0.2)	2.0
39	7	4	11 (1.3)	9.0	7	1	8 (0.9)	9.9	3	3	6 (0.7)	6.0
45	—	4	4 (0.5)	3.3	—	1	1 (0.1)	1.2	2	4	6 (0.7)	6.0
51	6	4	10 (1.1)	8.2	2	2	4 (0.4)	4.9	7	1	8 (0.9)	8.0
52	13	5	18 (2.0)	14.8	8	5	13 (1.4)	16	10	8	18 (2.0)	18.0
53	3	2	5 (0.6)	4.1	3	3	6 (0.7)	7.4	1	—	7 (0.8)	7.0
56	6	1	7 (0.8)	5.7	4	2	6 (0.7)	7.4	1	5	6 (0.7)	6.0
58	1	—	1 (0.1)	0.8	2	—	2 (0.2)	2.5	—	2	2 (0.2)	2.0
59	1	1	2 (0.2)	1.6	—	—	—		—	—	—	
66	—	1	1 (0.1)	0.8	1	—	1 (0.1)	1.2	2	2	4 (0.5)	4.0
68	—	3	3 (0.3)	2.5	2	—	2 (0.2)	2.5	4	1	5 (0.6)	5.0
Low risk												
6	5	3	8 (0.9)	6.6	—	2	2 (0.2)	2.5	4	2	6 (0.7)	6.0
11	—	—	—		—	—	—		—	1	1 (0.1)	1.0
40	—	—	—		—	1	1 (0.1)	1.2	1	1	2 (0.2)	2.0
42	—	—	—		1	—	1 (0.1)	1.2	—	—	—	
43	2	—	2 (0.2)	1.6	1	2	3 (0.3)	3.7	—	2	2 (0.2)	2.0
44	1	2	3 (0.3)	2.5	1	1	2 (0.2)	2.5	1	2	3 (0.3)	3.0
54	4	2	6 (0.7)	4.9	2	1	3 (0.3)	3.7	—	2	2 (0.2)	2.0
70	4	4	8 (0.9)	6.6	5	2	7 (0.8)	8.6	2	4	6 (0.7)	6.0
74	6	1	7 (0.8)	5.7	1	—	1 (0.1)	1.2	—	2	2 (0.2)	2.0

[a]The same woman can be counted more than once because of multiple infections. [b]Percentage over all women. [c]Percentage over HPV-positive cases.

In the 14 cervical cancer cases, single HPV 52 infection was detected in three and HPV 18 was also detected in three cases including one multiple infection with HPV 6. HPV 16 was detected two times, HPV 31, HPV 33 and HPV 39, HPV42 and HPV 45 were detected once. In one cancer case, HPV could not be detected.

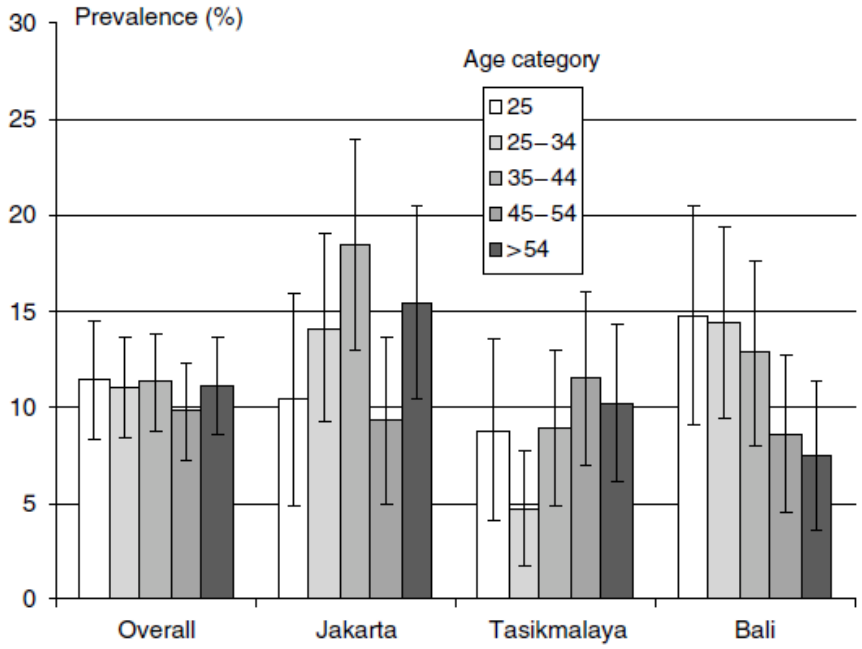


Figure 1 Age-specific prevalence of cervical human papillomavirus (HPV) DNA in percentages with 95% CI (Overall, Jakarta, Tasikmalaya and Bali, Indonesia).

The age-specific prevalence overall, and by region is shown in Figure 1. The overall age-specific prevalence in Indonesia was high in all ages (⩾9.8%), and ranged from 9.8 to 13.3%. In Jakarta, the overall prevalence was high (⩾9.3%), with two peaks: one of 18.4% in the 35–44-year age group and a second, of 15.4%, in women older than 54 years. The age-specific prevalence in Tasikmalaya peaked at 8.8% in the youngest age group of <25 years, declined to 4.7% in the 25–34-year group, and formed a second peak at 11.5% in the 45–54-year group. In Bali, HPV prevalence declined with age, from 14.7% in the <25-year group to 7.4% in the >54-year group, the trend in this decline was significant ($P<0.01$).

Risk factors for HPV infection

The association between HPV infection and characteristics of the studied women overall and by region after adjustment for age are shown in Table 2. Overall, being screened before was associated with a higher HPV positivity than never being screened before (OR 1.67, 95% CI 1.13–2.44) as was having had more than one partner than women having had one partner (OR 1.81, 95% CI 1.31–2.51). In Jakarta, HPV positivity was inversely associated with daily income when women having ⩾US

$3 per day were compared with those with ⩽US $1 per day (OR 0.51, 95% CI 0.28–0.92, with a significant trend of P=0.02), while having had more than one partner was associated with a higher HPV positivity than one partner (OR 2.66, 95% CI 1.64–4.33). In Tasikmalaya, the few divorced women had an OR of 6.0 (95% CI 1.39–25.91) with HPV positivity compared with married women. Although not significant, it seemed that nulliparous women were more often HPV positive (OR 5.35, 95% CI 0.92–31.08) than women with 1–2 children. In Bali, being 55 years or older was inversely associated with positivity (OR 0.46, 95% CI 0.23–0.96). In contrast with Jakarta, in Bali higher daily income was associated with higher positivity (OR 1.91, 95% CI 1.08–3.38, with a trend of P=0.03). In all three regions, HPV positivity was unrelated to having had a previous Pap smear, education level, smoking, the number of miscarriages, age at menarche, age at marriage, or first pregnancy.

Table 2 ORs for HPV detection and corresponding 95% CI

	Overall			Jakarta			Tasikmalaya			Bali		
	HPV neg	HPV pos	OR[a] (95% CI)	HPV neg	HPV pos	OR[a] (95% CI)	HPV neg	HPV pos	OR[a] (95% CI)	HPV neg	HPV pos	OR[a] (95% CI)
Age												
<25	365	47	1	112	13	1	125	12	1	128	22	1
25–34	509	63	0.96 (0.64–1.44)	165	27	1.41 (0.70–2.85)	183	9	0.51 (0.21–1.25)	161	27	0.98 (0.53–1.79)
35–44	494	76	1.20 (0.81–1.76)	155	35	1.95 (0.98–3.85)	175	17	1.01 (0.47–2.19)	164	24	0.85 (0.46–1.59)
45–54	495	54	0.85 (0.56–1.28)	156	16	0.88 (0.41–1.91)	170	22	1.35 (0.64–2.83)	169	16	0.55 (0.28–1.09)
⩾55	518	65	0.97 (0.65–1.45)	170	31	1.57 (0.79–3.13)	185	21	1.18 (0.56–2.49)	163	13	0.46 (0.23–0.96)
Trend	0.48			0.63			0.11			0.01		
Screened before												
No	2186	267	1	615	95	1	817	78	1	754	94	1
Yes	187	37	1.67 (1.13–2.44)	141	27	1.25 (0.76–2.05)	19	3	1.43 (0.41–5.00)	27	7	1.98 (0.83–4.75)
Salary												
<1$ per day	840	104	1	99	23	1	371	42	1	370	39	1
1–3$ per day	861	109	1.01 (0.76–1.35)	353	61	0.77 (0.45–1.30)	224	13	0.53 (0.27–1.01)	284	35	1.11 (0.68–1.81)
>3$ per day	583	75	1.03 (0.75–1.42)	257	31	0.51 (0.28–0.92)	214	21	0.82 (0.47–1.45)	112	23	1.91 (1.08–3.38)
Trend	0.80			0.02			0.41			0.03		
Marital status												
Married	2260	287	1	683	111	1	795	74	1	783	102	1
Divorced	10	4	2.96 (0.92–9.55)	5	1	1.12 (0.13–9.85)	5	3	6.0 (1.38–25.91)	0	0	0
Widowed	109	14	1.07 (0.59–1.93)	70	10	0.85 (0.40–1.80)	39	4	0.9 (0.31–2.64)	0	0	0
Age at marriage												
<16	287	42	1	96	21	1	144	16	1	47	5	1
16–18	449	56	0.85 (0.55–1.30)	218	33	0.70 (0.38–1.28)	325	31	0.92 (0.48–1.74)	217	34	1.49 (0.55–4.03)
19–21	535	62	0.79 (0.52–1.20)	242	32	0.60 (0.33–1.10)	224	21	0.96 (0.48–1.92)	328	37	1.21 (0.44–3.28)
>21	1104	145	0.89 (0.62–1.29)	202	36	0.79 (0.43–1.45)	144	13	0.92 (0.42–2.02)	188	26	1.37 (0.49–3.87)
Trend	0.67			0.64			0.61			0.84		
Number of marriages												
1	2112	250	1	675	92	1	687	61	1	750	97	1
>1	261	55	1.81 (1.31–2.51)	82	30	2.66 (1.64–4.33)	149	20	1.40 (0.81–2.43)	30	5	1.51 (0.56–4.06)
Parity												
0	22	3	1.03 (0.30–3.50)	9	1	0.76 (0.09–6.23)	4	2	5.34 (0.92–31.06)	9	0	0
1/2	1063	138	1	302	41	1	333	33	1	428	64	1
3/4	719	87	0.91 (0.67–1.25)	233	47	1.43 (0.84–2.41)	272	20	0.60 (0.31–1.15)	214	20	0.71 (0.40–1.28)
⩾5	455	58	0.99 (0.66–1.47)	172	23	0.96 (0.50–1.85)	175	25	1.09 (0.54–2.20)	108	10	0.82 (0.35–1.90)
Trend	0.83			0.73			0.51			0.11		

OR = odds ratio, CI = confidence interval. Figures do not add up to the total because of missing values. [a]Adjusted for age.

Discussion

Among a mainly unscreened population of women in Indonesia, we found an intermediate overall prevalence of HPV, highest types 52, 16, and 18, with a different age specific pattern in the three regions.

Clifford *et al* defined age-standardized HPV prevalence rates as 'low', 'intermediate', and 'high'. Using a previous classification, the overall prevalence found (11.3%) was intermediate (3), comparable to other Asian countries like Thailand (Lampang, 9.1%), Vietnam (Ho Chi Minh City, 10.6%), India (17.7%), and with South American countries such as Chile (14.0%) and Mexico (14.5%) (12-16). Cervical cancer incidence is related to HPV prevalence in the region and the presence of organised screening, among other factors (17) and that estimated for Indonesia, a country without organised screening (at least 30 per 100 000 women per year) is consistent with recorded HPV (5,2) prevalence.

Areas of high HPV prevalence and with no decline in older age groups all have high incidence and mortality and very low-income levels (4), as is true for Jakarta and Tasikmalaya.

In contrast, in Bali a significant decline in HPV prevalence with increasing age was seen, as in some western countries, in Korea (4) and among Hindu women in India (18). In the latter, the reproductive period (and thus active sexual life) of Hindu women mostly ends by the age of 30–35 years, which could explain the decreasing prevalence observed. Genetic factors in the susceptibility to HPV infection in older age could not be excluded. Unfortunately, we lack such information for Hindu women in our study.

Two potential selection biases could have occurred: we attempted to screen at least 80% of all women aged 20–65 in the villages who were visited by the screening programme. All women were informed at their homes but only the women who actually answered the call to participate were included. The percentage of targeted women included was only available for Bali, (83.7%). Because the method of selection was similar in all regions, and because all households in an area were visited, we assume that participation is similar to that in Bali. The major reasons for non-participation were having to work, sickness or anxiety. The second bias concerns the fact that culturally and religiously, sexual intercourse is not allowed before marriage in Indonesia. For this reason, a few questions were believed to be inappropriate and thought not to be answered honestly. Instead, we asked acceptable questions: '*age of first marriage*' and '*number of marriages*'. We did not perform gynaecological examination on unmarried women who would strictly still be virgin; all our screened women were married, divorced or widowed.

HPV 52 was the most prevalent type in Jakarta and Bali, and the second most prevalent type (after 18) in Tasikmalaya accounting, respectively, for 23.1, 20.1, and

23.4% of the high-risk positive samples. Worldwide, high prevalence of HPV 52 is also reported from China, Taiwan, and Costa Rica (19-21). It was also detected, in 21.4% (three of 14) of the cervical cancers as it was in 14% of cases in another study (5). Clifford *et al* (22) also identified HPV 52 more frequently in cervical cancer in Asia than in other parts of the world. Adding HPV 52, to types 16 and 18, in a prophylactic HPV vaccine when introduced in Indonesia would therefore seem appropriate.

HPV 16 prevalence in this population was comparable to that in most parts of the world (3), though that of HPV 18 was higher than regions with comparable overall HPV prevalence (12,13,14,15,16). HPV 18 accounted for a significant population of high-risk HPV-positive samples: 10.3, 22.6, and 15.6%, respectively, for Jakarta, Tasikmalaya, and Bali. In most countries, HPV 16 is by far the most prevalent type in cervical carcinoma and in the general population, followed by HPV 18 (3,22). Although we found small inter-regional differences (see Table 1), HPV 18 was as common as 16, reflecting their predominant roles in cervical cancer in Indonesia (5,6). HPV 39 was prevalent in the general population in this study, but it is rare in cervical carcinomas in Indonesia and other parts of Asia (5,22).

As expected, a history of more than one sexual partner was associated with HPV positivity (23,24). A small group of divorced women in Tasikmalaya and women with high daily income in Bali were associated with HPV positivity, probably reflecting their sexual behaviour and that of their partners (23). Unfortunately, little relevant information is available because sexuality is still a taboo subject in Indonesia; more research could be revealing. Overall, previous screening was associated with higher HPV positivity. Possibly, reflecting earlier symptoms.

In conclusion, in Indonesia HPV 16 and 18 are equally common in the general population, as they are in cervical cancer. HPV 52 was the most prevalent type, suggesting that it should also be included in the prophylactic HPV vaccine when introduced in Indonesia.

Acknowledgements

This study was funded by the Female Cancer Foundation, Leiden University Medical Center, the Netherlands (www.femalecancerfoundation.org).

References

1. Tjindarbumi D, Mangunkusumo R. Cancer in Indonesia, present and future. Japanese journal of clinical oncology. 2002;32 Suppl:S17-21. Epub 2002/04/18.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA: a cancer journal for clinicians. 2005;55(2):74-108. Epub 2005/03/12.
3. Clifford GM, Gallus S, Herrero R, Munoz N, Snijders PJ, Vaccarella S, et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet (London, England). 2005;366(9490):991-8. Epub 2005/09/20.
4. Franceschi S, Herrero R, Clifford GM, Snijders PJ, Arslan A, Anh PT, et al. Variations in the age-specific curves of human papillomavirus prevalence in women worldwide. Int J Cancer. 2006;119(11):2677-84. Epub 2006/09/23.
5. Schellekens MC, Dijkman A, Aziz MF, Siregar B, Cornain S, Kolkman-Uljee S, et al. Prevalence of single and multiple HPV types in cervical carcinomas in Jakarta, Indonesia. Gynecologic oncology. 2004;93(1):49-53. Epub 2004/03/30.
6. Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. Journal of the National Cancer Institute. 1995;87(11):796-802. Epub 1995/06/07.
7. de Boer MA, Vet JN, Aziz MF, Cornain S, Purwoto G, van den Akker BE, et al. Human papillomavirus type 18 and other risk factors for cervical cancer in Jakarta, Indonesia. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2006;16(5):1809-14. Epub 2006/10/03.
8. Kleter B, van Doorn LJ, Schrauwen L, Molijn A, Sastrowijoto S, ter Schegget J, et al. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. Journal of clinical microbiology. 1999;37(8):2508-17. Epub 1999/07/16.
9. Longuet M, Cassonnet P, Orth G. A novel genital human papillomavirus (HPV), HPV type 74, found in immunosuppressed patients. Journal of clinical microbiology. 1996;34(7):1859-62. Epub 1996/07/01.
10. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. The New England journal of medicine. 2003;348(6):518-27. Epub 2003/02/07.
11. Segi M, Fujisaku S, Kurihara M, Narai Y, Sasajima K. The age-adjusted death rates for malignant neoplasms in some selected sites in 23 countries in 1954-1955 and their geographical correlation. The Tohoku journal of experimental medicine. 1960;72:91-103. Epub 1960/08/25.
12. Lazcano-Ponce E, Herrero R, Munoz N, Cruz A, Shah KV, Alonso P, et al. Epidemiology of HPV infection among Mexican women with normal cervical cytology. Int J Cancer. 2001;91(3):412-20. Epub 2001/02/15.
13. Pham TH, Nguyen TH, Herrero R, Vaccarella S, Smith JS, Nguyen Thuy TT, et al. Human papillomavirus infection among women in South and North Vietnam. Int J Cancer. 2003;104(2):213-20. Epub 2003/02/06.
14. Sukvirach S, Smith JS, Tunsakul S, Munoz N, Kesararat V, Opasatian O, et al. Population-based human papillomavirus prevalence in Lampang and Songkla, Thailand. The Journal of infectious diseases. 2003;187(8):1246-56. Epub 2003/04/16.

15. Ferreccio C, Prado RB, Luzoro AV, Ampuero SL, Snijders PJ, Meijer CJ, et al. Population-based prevalence and age distribution of human papillomavirus among women in Santiago, Chile. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2004;13(12):2271-6. Epub 2004/12/16.
16. Franceschi S, Rajkumar R, Snijders PJ, Arslan A, Mahe C, Plummer M, et al. Papillomavirus infection in rural women in southern India. British journal of cancer. 2005;92(3):601-6. Epub 2005/01/26.
17. Bosch FX, de Sanjose S. Chapter 1: Human papillomavirus and cervical cancer--burden and assessment of causality. Journal of the National Cancer Institute Monographs. 2003(31):3-13. Epub 2003/06/17.
18. Duttagupta C, Sengupta S, Roy M, Sengupta D, Bhattacharya P, Laikangbam P, et al. Are Muslim women less susceptible to oncogenic human papillomavirus infection? A study from rural eastern India. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2004;14(2):293-303. Epub 2004/04/17.
19. Herrero R, Castle PE, Schiffman M, Bratti MC, Hildesheim A, Morales J, et al. Epidemiologic profile of type-specific human papillomavirus infection and cervical neoplasia in Guanacaste, Costa Rica. The Journal of infectious diseases. 2005;191(11):1796-807. Epub 2005/05/05.
20. Jeng CJ, Phdl, Ko ML, Ling QD, Shen J, Lin HW, et al. Prevalence of cervical human papillomavirus in Taiwanese women. Clinical and investigative medicine Medecine clinique et experimentale. 2005;28(5):261-6. Epub 2005/11/04.
21. Dai M, Bao YP, Li N, Clifford GM, Vaccarella S, Snijders PJ, et al. Human papillomavirus infection in Shanxi Province, People's Republic of China: a population-based study. British journal of cancer. 2006;95(1):96-101. Epub 2006/06/15.
22. Clifford GM, Smith JS, Plummer M, Munoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. British journal of cancer. 2003;88(1):63-73. Epub 2003/01/31.
23. Vaccarella S, Franceschi S, Herrero R, Munoz N, Snijders PJ, Clifford GM, et al. Sexual behavior, condom use, and human papillomavirus: pooled analysis of the IARC human papillomavirus prevalence surveys. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006;15(2):326-33. Epub 2006/02/24.
24. Vaccarella S, Herrero R, Dai M, Snijders PJ, Meijer CJ, Thomas JO, et al. Reproductive factors, oral contraceptive use, and human papillomavirus infection: pooled analysis of the IARC HPV prevalence surveys. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006;15(11):2148-53. Epub 2006/11/23.

Chapter 4

Single-visit approach of cervical cancer screening: see and treat in Indonesia

JNI Vet, JL Kooijman, FC Henderson, FM Aziz, G Purwoto, H Susanto, I G D Surya, S Budiningsih, S Cornain, GJ Fleuren, JB Trimbos, AAW Peters

Adapted from British Journal of Cancer (2012) 107 (5), 772-777

Abstract

Background: We performed a cross-sectional study in Indonesia to evaluate the performance of a single-visit approach of cervical cancer screening, using visual inspection with acetic acid (VIA), histology and cryotherapy in low-resource settings.

Methods: Women having limited access to health-care facilities were screened by trained doctors using VIA. If the test was positive, biopsies were taken and when eligible, women were directly treated with cryotherapy. Follow-up was performed with VIA and cytology after 6 months. When cervical cancer was suspected or diagnosed, women were referred. The positivity rate, positive predictive value (PPV) and approximate specificity of the VIA test were calculated. The detection rate for cervical lesions was given.

Results: Screening results were completed in 22 040 women, of whom 92.7% had never been screened. Visual inspection with acetic acid was positive in 4.4%. The PPV of VIA to detect CIN I or greater and CIN II or greater was 58.7% and 29.7%, respectively. The approximate specificity was 98.1%, and the detection rate for CIN I or greater was 2.6%.

Conclusion: The single-visit approach cervical cancer screening performed well, showing See and Treat is a promising way to reduce cervical cancer in Indonesia.

Keywords: single-visit approach; visual inspection with acetic acid (VIA); cryotherapy; Indonesia

Introduction

Cervical cancer is the second most common cancer among women in developing countries, where more than 85% of the 530 000 new cervical cancer cases occur worldwide (1). Cervical cancer rates in Indonesia are high, but because there is no population-based cancer registration, reports on the incidence of cervical cancer and efficacy of opportunistic screening procedures are limited. The governmental hospitals report cervical cancer to be up to 28% among all female cancer cases, representing 75% of all gynaecological cancers that are mostly diagnosed in advanced stages (2,3).

In developed countries, introduction of organised Pap smear screening led to tremendous drops in cervical cancer rates, as it allows detection and treatment of precancerous lesions and early-stage cervical cancer (4-8). Unfortunately, the health-care infrastructure in developing countries often prohibits successful implementation of organised conventional cytological screening due to lack of financial support, professional human resources and laboratory services (9,10).

For many years, research has been done to develop alternative screening 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 (10-15). 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 (16).

This research describes a single-visit approach cervical cancer screening programme using VIA and cryotherapy in three areas in Indonesia, the first of its kind to be systematically performed and described in this setting. The main objective was to study the performance of this approach, targeting high-risk women aged 20–60 years, living in low-resource circumstances with limited access to health-care facilities. Information on prevalence of premalignant cervical cancer lesions and cervical cancer was collected and the performance of VIA was compared with histology, the reference diagnosis. When the VIA test was positive and women were eligible, they were directly treated with cryotherapy and registered for follow-up with VIA and cytology after 6 months. As follow-up was scheduled after therapy, the cure rate of cryotherapy could be measured.

The approval for this study was given by the institutional review boards of the three collaborating University Hospitals Cipto Mungunkusumo in Jakarta, Hasan Sadikin in Bandung, Sanglah Bali and the regional hospital in Tasikmalaya.

Methods

Study design

This community-based, single-visit approach, cervical cancer prevention programme was performed from October 2004 to June 2006. The programme was organised in collaboration with the University of Indonesia and the Cipto Mangunkusumo Hospital in Jakarta, the Padjadjaran University and the Hasan Sadikin Hospital in Bandung, Siliwangi University and General Hospital in Tasikmalaya, Udayana University and the Sanglah Hospital in Denpasar in Indonesia, and the Leiden University Medical Center in the Netherlands. This project aimed to screen women living in low-resource areas with limited access to health-care facilities. Screening was free of charge. Three mobile clinics with teams consisting of two doctors, two nurses, two public health nurses and a driver visited low-resource areas in Jakarta and Tasikmalaya (Java) and in rural areas on the island of Bali. All the members of the mobile clinic teams were trained in a 1-week central training course. In addition, the general practitioners (Jakarta and Tasikmalaya) and residents in gynaecology (Bali) were specially trained in VIA and cryotherapy and performed the screening using VIA. If the test was positive, biopsies were taken and women were directly treated with cryotherapy and registered for follow-up after 6 months. When cervical cancer was suspected or diagnosed, women were referred to one of the three collaborating regional University Hospitals for further evaluation and treatment. All costs for treatment, transportation and costs for an accompanying relative were covered by the Female Cancer Foundation.Figure 1 shows a flowchart of the study design.

Next to the screening through VIA, we performed conventional cytological screening in the same setting. However, in this paper, we focus on the VIA screening procedure; therefore, the cytological procedure and results will not be discussed.

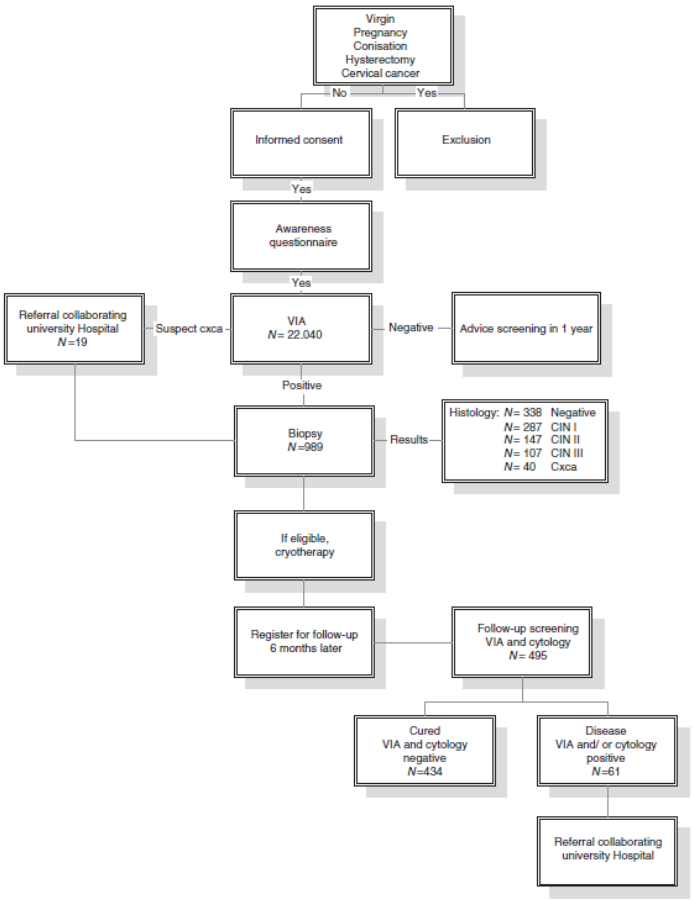


Figure 1 Flowchart of the study design.

Participants

The target group was women aged 20–60 years of whom we tried to reach 80% in each region. Women were excluded if they were virgin, pregnant, did not have an intact cervix or had a history of cervical cancer. To reach women for enrolment, collaboration was set up with the 'Pembinaan Kesejahteraan Keluarga' (PKK), the national Indonesian family welfare organisation that has access to the smallest villages. In the selected areas, the members of this local PKK encouraged women to participate in the project by visiting them in their homes, informing them about risk factors, prevention, early detection and treatment of cervical cancer. Printed information designed in collaboration with the Indonesian cancer foundation, 'Yayasan Kanker Indonesia' (YKI), was given to the women.

On scheduled days, the mobile clinic team arrived and set up a screening site in existing public facilities, mostly in small health facilities. Public health nurses counselled the women individually. After describing and explaining the procedure of screening and possible treatment, informed consent was obtained. Women were interviewed about cervical cancer risk factors, reproductive health and sociodemographic issues.

Test methods

With an adequate light source, the cervix was inspected visually. With a cotton swap, a 5% diluted acetic acid solution was applied to the cervix for 1 min. After application, acetowhite lesions in the transformation zone, close to the squamocolumnar junction, were considered positive and eligible for treatment (17). Cervicitis, nabothian cysts and polyps were considered negative. Biopsy was taken of all VIA-positive lesions and polyps were removed; both specimens were sent to the Department of Pathology of the collaborating University Hospital for diagnosis.

When the VIA result was negative, subsequent screening was advised at 1 year, according to the Indonesian guidelines. When the screening test was positive, women were counselled for immediate cryotherapy.

Cryotherapy was considered if the lesion was not suspected for cervical cancer, did not extend over more than 75% of the cervix, into the cervical canal or vaginal wall, and could be covered fully by the cryotherapy probe. Cryotherapy was provided in cycles of 3 min of freeze, 3 min of thaw and 3 min of freeze with an Erbe cryogun (Tuebingen, Germany) using a cone-shaped probe and CO_2 gas (Jakarta and Tasikmalaya) or N_2O (Bali). After cryotherapy, women received non-narcotic analgesics and one oral dose of antibiotics, according to the Indonesian guidelines. They were informed about side effects and instructed not to have sexual intercourse for 4 weeks and to return to the health centre in case of severe abdominal pain, fever (>38 °C) and purulent or bloody discharge.

In case of cervicitis, antibiotics were administered. Among women with a suspected diagnosis of cervical cancer based on clinical evaluation, biopsy was taken and they were referred to the collaborating University Hospitals for further diagnosis, staging and treatment. All histologic specimens were processed and diagnosed by the pathologists in the collaborating University Hospitals.

Table 1 Characteristics of the participants

	Overall	Jakarta	Tasik	Bali
Number	$n=22040$	$n=7480$	$n=8007$	$n=6553$
Characteristic		**Numbers (%)**		
Age				
<19	151 (0.7)	47 (0.6)	44 (0.5)	60 (0.9)
20–29	4652 (21.1)	1545 (20.7)	1535 (19.2)	1572 (24.0)
30–39	8169 (37.1)	2642 (35.3)	3133 (39.1)	2394 (36.5)
40–49	6006 (27.3)	2151 (28.8)	2346 (29.3)	1509 (23.0)
50–59	2607 (11.8)	968 (12.9)	808 (10.1)	831 (12.7)
>60	351 (1.6)	125 (1.7)	136 (1.7)	90 (1.4)
Missing	104 (0.5)	2 (0.0)	5 (0.1)	97 (1.5)
Ever screened before				
No	20449 (92.8)	6359 (85.0)	7868 (98.3)	6222 (94.9)
Yes	1490 (6.8)	1101 (14.7)	124 (1.5)	265 (4.0)
Missing	101 (0.4)	20 (0.3)	15 (0.2)	66 (1.0)
Education				
Illiterate	2096 (9.5)	317 (4.2)	13 (0.2)	1766 (26.9)
Primary school	9293 (42.2)	2773 (37.1)	3771 (47.1)	2749 (42.0)
High school	8709 (39.5)	3747 (50.1)	3183 (39.8)	1779 (27.1)
>High school	1907 (8.7)	635 (8.5)	1036 (12.9)	236 (3.6)
Missing	35 (0.1)	8 (0.1)	4 (0.0)	23 (0.4)
Income per day				
<1 US $ per day	7367 (33.4)	1016 (13.6)	3504 (43.8)	2847 (43.4)
1–3 US $ per day	7972 (36.2)	3557 (47.6)	1981 (24.7)	2434 (37.1)
>3 US $ per day	6092 (27.6)	2667 (35.7)	2306 (28.8)	1119 (17.1)
Missing	609 (2.8)	240 (3.2)	216 (2.7)	153 (2.3)
Marital status				
Not married yet	15 (0.1)	0 (0.0)	5 (0.1)	10 (0.2)
Married	21313 (96.7)	7090 (94.8)	7704 (96.2)	6519 (99.5)
Separated	134 (0.6)	67 (0.9)	67 (0.8)	0 (0.0)
Widowed	545 (2.5)	315 (4.2)	225 (2.8)	5 (0.1)
Missing	33 (0.1)	8 (0.1)	6 (0.1)	19 (0.2)
Number of children				
0	880 (4.0)	360 (4.8)	318 (4.0)	202 (3.1)
1/2	9513 (43.2)	2766 (37.0)	3139 (39.2)	3608 (55.1)
3/4	7606 (34.5)	2555 (34.2)	3028 (37.8)	2023 (30.9)
5/6	2754 (12.5)	1143 (15.3)	1095 (13.7)	516 (7.9)
>7	1211 (5.5)	649 (8.7)	394 (4.9)	168 (2.6)
Missing	76 (0.3)	7 (0.1)	33 (0.4)	36 (0.5)
Mean (s.d.)				
Menarche	13.9 (1.5)	13.7 (1.7)	14.1 (1.5)	13.9 (1.3)
Age at marriage	19.9 (3.7)	20.0 (4.1)	19.4 (3.6)	20.7 (3.2)
Age at first child	21.1 (4.1)	21.2 (4.5)	21.1 (3.8)	20.9 (4.0)
Sexual partners	1.1 (0.4)	1.2 (0.5)	1.2 (0.5)	1.0 (0.2)

Follow-up 6 months after cryotherapy was scheduled at the initial screening. Women were informed about the date for their return appointment and received a follow-up card with the information. The mobile clinic came down to the village at the set date, if a patient did not show up at the follow-up appointment, she was contacted by phone or by the PKK.

The follow-up screening consisted of cytological and repeat VIA screening. The Pap smears were taken on the site and were diagnosed at the collaborating hospitals. In the follow-up, the cytopathologists reading the slides were aware of the VIA test results.

Patients were considered cured when both cytological screening and their VIA test were negative on follow-up. Cure rate was calculated by dividing the number of women who were negative on cytological and VIA follow-up screening by the number of women screened in the follow-up.

Treatment failure was diagnosed if at least one test was positive; these patients were referred to the collaborating University Hospitals for colposcopy.

During this study, site visits to guard the screening process and VIA quality were performed regularly in all three regions.

Calculations and data management

The positive predictive value (PPV) of the VIA test was calculated by dividing the histology-proven cases by the VIA test positive cases. The detection rate of CIN I, II, III and cervical cancer was calculated by dividing the number of screened women with these lesions by the total number of screened women. The specificity of the VIA test could not be given as there were no biopsies taken of the women who were negative on VIA screening but an approximate specificity of the VIA test was calculated following the example described by Sankaranarayanan *et al* (17) by dividing the number of women with negative screening tests by the total number screened subjects minus the number of true-positive cases detected by the test.

Data management was performed using Microsoft Access 2003 and SPSS software (SPSS, version 17, SPSS Inc., Chicago, IL, USA).

Results

Demographics

Test results were completed in 22 040 women, all screened using VIA. Of these, 7480 (33.9%) were from Jakarta, 8007 (36.3%) from Tasikmalaya and 6553 (29.7%) from Bali. Mean age was 37.5 years (range 12–70) and 64.4% were age 30–49. Overall, 92.7% of women had never been screened before and one-third lived beneath the WHO poverty border of 1 US dollar a day. Table 1 reports participant characteristics.

Screening

Of the 22 040 total women, 970 (4.4%) were VIA positive and 19 (0.1%) were suspected to have cervical cancer. The VIA positivity rate varied within the geographic regions from 2.3% in Jakarta to 3.9% in Bali and 6.8% in Tasikmalaya. A descending

trend was seen when VIA positivity rate was calculated over time; when the total amount of women was divided in four even parts, the VIA positivity rate decreased from 5.3%within the first 25% of the screened women, to 4.8% in the second 25%, to 4.6% in the third 25%, finally, to a positivity rate of 3.3% within the last 25% of the screened women.

On histologic analysis, 338 (34.2%) had a normal histology, 287 (1.3%) had a diagnosis of CIN I, 147 (0.7%) CIN II, 105 (0.5%) CIN III and 42 (0.2%) adenocarcinoma *in situ*, adenocarcinoma, or squamous cell carcinoma. In 7% of the biopsies (70/989), there was no diagnosis as the quality of the biopsies was too poor for histological classification. The overall detection rates for lesions CIN I and CIN II were 2.6% and 1.3%, respectively.

Most positive results were in the 30–39-year-old age group, followed by the 40–49-year-old age group. Together, these groups account for 70.7% of VIA-positive cases and 73.0% of histologic-positive cases. For details on age distribution and positive test results and (pre)malignant detection rate, see Table 2.

The overall PPV of VIA for a histological diagnosis of CIN I or greater was 581/989 × 100%=58.7%, and for a histological diagnosis of CIN II or greater was 294/989 × 100%=29.7%. The approximate specificity was 21051/(22040−581) × 100%=98.1%.

Cervical malignancy was based on the histological specimen. The 40 cases of cervical cancer included 38 squamous cell carcinomas and 2 adenocarcinomas. All the 40 cases were referred to the collaborating University Hospitals.

Table 2 VIA positivity and (pre)malignancy detection rate

	No. screened	VIA+ Number (%)	CIN I	CIN II	CIN III	Cxca
			Number (detection rate in %)			
Overall	22 040	989 (4.5)	287 (1.3)	147 (0.7)	107 (0.5)	40 (0.2)
<19	151	3 (2.0)	1 (0.7)	0 (0.0)	1 (0.7)	0 (0.0)
20–29	4652	174 (3.7)	46 (1.0)	25 (0.5)	26 (0.6)	1 (0.02)
30–39	8169	412 (5.0)	129 (1.6)	63 (0.8)	40 (0.5)	8 (0.1)
40–49	6006	282 (4.7)	86 (1.4)	46 (0.8)	27 (0.4)	14 (0.2)
50–59	2607	84 (3.2)	21 (0.8)	8 (0.3)	9 (0.3)	6 (0.2)
>60	351	22 (6.3)	1 (0.3)	4 (1.1)	3 (0.9)	10 (2.8)
Age unknown	104	12 (11.5)	3 (2.9)	1 (1.0)	1 (1.0)	1 (1.0)

VIA-positive patients including suspect for malignancy. CIN III including 2 cases of adenocarcinoma *in situ*, 1 in the 20–29 and 1 in the 40–49 age group and including 18 cases diagnosed as carcinoma *in situ*. Cxca: cervical carcinoma, including micro- and macroinvasive squamous cell carcinoma and adenocarcinoma.

Treatment

Based on positive VIA, 918 women were to receive cryotherapy. Most of these women received treatment directly, some after consultation with their husband or family.

Although during and directly after the cryotherapy, women had some abdominal cramps, dizziness, flushing and sometimes fainting, no major side effects such as severe pelvic cramps, severe bleeding, anaphylactic reactions or pelvic inflammatory disease were reported.

Follow-up

Follow-up screening consisted of cytologic and repeat VIA screening 6 months after cryotherapy. Of the 989 women that were VIA positive, 71 women were excluded from follow-up as they were referred; 19 women were clinically suspect of cervical cancer, 25 women were diagnosed cervical cancer on histology, 18 women were diagnosed with carcinoma *in situ*, 2 with adenocarcinoma *in situ* and 7 women were referred based on other reasons than suspect cervical cancer.

The follow-up screening results are shown in Table 3. Overall, 495 of the 918 scheduled women (53.9%) received follow-up screening. Of these women, 92.0% was diagnosed 'cured', based on negative cytological and negative VIA screening. The cure rate decreased when the severity of the initial cervical lesion increased.

Overall, in 43 women, the cytological results in follow-up screening were positive, these consisted from 3 × ASCUS, 39 LSIL and 1 × HSIL on Bethesda classification.

Women with positive VIA and/or an abnormal result in cytology at follow-up screening were referred to the collaborating University Hospitals for further investigations.

Table 3 Follow-up on VIA-positive women

	VIA+	Number FU	FU VIA−	FU VIA+	Missing	FU Cyt−	FU Cyt+	Missing	Cyt and VIA neg	Cure rate (%)
Overall	918	495	471	20	4	450	43	2	434	92.0
Histology neg	336	147	141	6	0	143	3	1	139	
CIN I	285	177	171	5	1	165	12	0	160	93.6
CIN II	147	93	88	3	2	78	15	0	75	85.2
CIN III	81	56	50	5	1	43	12	1	40	80.0
No result	69	22	21	1	0	21	1	0	20	95.2

VIA + are the women who received cryotherapy based on VIA-positive results. Number FU are the women that actually underwent follow-up screening. Missing results are given for VIA and cytology.

Discussion

In three essentially different areas in Indonesia, the metropolitan Jakarta, the city and rural areas of Tasikmalaya, and the city and rural areas on the island of Bali, we studied the performance of the concept of single-visit approach cervical cancer screening using VIA, histology and cryotherapy.

Collaboration with existing health-care services, the Indonesian family welfare movement (PKK) and the Indonesian cancer foundation (YKI), proved to be efficient in

setting up an infrastructure to reach women in the villages and to mobilise them for participating in the screening programme. The targeted population was reached, 92.7% of participants had never been screened before, 64.4% were in the high-risk age group of 30–49 years, and one-third lived below the WHO poverty border. All women attending the programme were informed about cervical cancer and prevention and underwent the screening procedures. Of the women with abnormal results scheduled for cryotherapy, most of these women directly received treatment, some after consultation with their husband or family. In conclusion, after 4 months of preparation, the screening programme and the precedent awareness programme could be adapted and implemented in the local system.

Our study gives insight in the use of VIA in the field conditions and its routine performance in health services in Indonesia. In this unscreened population, the overall prevalence of cervical cancer found was high, being 40 cervical cancer cases in 22 040 women. The overall VIA positivity rate, 4.4%, seemed rather low. During this study, we concentrated on reaching the target group and on the quality of the VIA screening. Regarding the characteristics of the studied population and the findings on the site visits, we do think these are representative. Although we expected to find higher positivity rates, our rates are comparable to population-based VIA screening programs in high incidence areas as Tanzania (VIA positivity 3.8%), Bangladesh (4.8%) and Angola (6.6%) (18-20).

The high rate of VIA positivity in Tasikmalaya compared with Jakarta and Bali could be explained by epidemiological differences. The percentage of women that had never been screened was higher in Tasikmalaya. Moreover, promiscuity seems to be more common in Tasikmalaya as there is more labour migration than in Jakarta and Bali.

The highest prevalence of positive VIA was found in the high-risk group of women aged 30–49, accounting for 70.7% of positive cases. The overall PPV of VIA for histological diagnosis of CIN I or greater and CIN II or greater was 58.7% and 29.3%, respectively. The resultant overtreatment rates are 41.3% and 70.7%, respectively. We think these overtreatment rates are acceptable as the morbidity associated with cryotherapy is low and the overall benefit of treatment in reducing the risk of cervical cancer in high incidence areas is significant (21). There were no major side effects reported, although we might not have been fully informed as follow-up rates are limited. However, these findings are consistent with earlier performed studies on the safety, acceptability and feasibility of cryotherapy, where side effects were found to be rare (22,14).

The set up of our study is limited in further objectifying the accuracy of VIA other than the PPV, as it lacks the necessary data to calculate sensitivity, specificity and negative predictive value. We calculated the approximate specificity (23), which was found to be 98.1%. A review on VIA test by Gaffikin *et al* (14) reported a specificity

range of 64–98% with a weighted mean of 83%. The fact that our approximate specificity is high can be explained by the low VIA positivity rate in our study (24).

The cure rate of the lesions 6 months after cryotherapy based on negative VIA and negative cytological follow-up screening in this study was 92.0%. As these cure rates are not based on histological diagnosis, they give an indication of cure in this study rather than being an absolute number. It must be taken into account that only 47.4% of the initial population that received cryotherapy came for follow-up screening, which may have led to a bias in these numbers.

Several conclusions can be drawn from our data. First, collaboration with existing health services in Indonesia, the Indonesian family welfare movement and the Indonesian cancer foundation, succeeded in reaching high-risk women having limited access to health-care facilities and living in low-resource settings. Further targeting coverage to reach high-risk women must be taken into account when new cost-effective screening programme policies are designed and expanded to other regions in Indonesia. Second, the use of VIA and cryotherapy performed well in this single-visit approach, and the acceptance of the therapy was high.

The cervical screening programme continued in all three areas. First, the programme was granted by the 'Goede doelen van de Nederlandse Postcode Loterij' through the Female Cancer Foundation. Granted by the Dutch Ministry of Foreign Affairs, the programme expanded from the original three regions described in this project to another five regions in Indonesia in North Sumatra, South Kalimantan and North Sulawesi. Now the Indonesian Ministry of Health and the Indonesian cancer Association 'Yayasan Kanker Indonesia' took over in cooperation with the faculties of Medicine in the collaborating regions.

This cross-sectional study showed that the See and Treat single-visit approach is a promising way to reduce cervical cancer in Indonesia.

Acknowledgements

This study was funded by the Female Cancer Foundation, Leiden University Medical Center, the Netherlands (www.femalecancerfoundation.org).

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. *CA Cancer J Clin* 61(2): 69–90
2. Tjindarbumi D, Mangunkusumo R (2002) Cancer in Indonesia, present and future. *Jpn J Clin Oncol*32(Suppl): S17–S21
3. Aziz MF (2009) Gynecological cancer in Indonesia. *J Gynecol Oncol* 20(1): 8–10
4. Hakama M, Chamberlain J, Day NE, Miller AB, Prorok PC (1985) Evaluation of screening programmes for gynaecological cancer. *Br J Cancer* 52(4): 669–673
5. WHO Meeting (1986) Control of cancer of the cervix uteri. A WHO meeting. *Bull World Health Organ*64(4): 607–618
6. Laara E, Day NE, Hakama M (1987) Trends in mortality from cervical cancer in the Nordic countries: association with organised screening programmes. *Lancet* 1(8544): 1247–1249
7. Anderson GH, Boyes DA, Benedet JL, Le Riche JC, Matisic JP, Suen KC, Worth AJ, Millner A, Bennett OM (1988) Organisation and results of the cervical cytology screening programme in British Columbia, 1955–85. *Br Med J (Clin Res Ed)* 296(6627): 975–978
8. Sankaranarayanan R, Budukh AM, Rajkumar R (2001) Effective screening programmes for cervical cancer in low- and middle-income developing countries. *Bull World Health Organ* 79(10): 954–962
9. Vizcaino AP, Moreno V, Bosch FX, Munoz N, Barros-Dios XM, Borras J, Parkin DM (2000) International trends in incidence of cervical cancer: II. Squamous-cell carcinoma. *Int J Cancer* 86(3): 429–435
10. Sankaranarayanan R, Rajkumar R, Theresa R, Esmy PO, Mahe C, Bagyalakshmi KR, Thara S, Frappart L, Lucas E, Muwonge R, Shanthakumari S, Jeevan D, Subbarao TM, Parkin DM, Cherian J (2004) Initial results from a randomized trial of cervical visual screening in rural south India. *Int J Cancer* 109(3): 461–467
11. University of Zimbabwe/JHPIEGO Cervical Cancer Project (1999) Visual inspection with acetic acid for cervical-cancer screening: test qualities in a primary-care setting. *Lancet* 353(9156): 869–873
12. Denny L, Kuhn L, Pollack A, Wainwright H, Wright TC (2000) Evaluation of alternative methods of cervical cancer screening for resource-poor settings. *Cancer* 89(4): 826–833
13. Mandelblatt JS, Lawrence WF, Gaffikin L, Limpahayom KK, Lumbiganon P, Warakamin S, King J, Yi B, Ringers P, Blumenthal PD (2002) Costs and benefits of different strategies to screen for cervical cancer in less-developed countries. *J Natl Cancer Inst* 94(19): 1469–1483
14. Gaffikin L, Blumenthal PD, Emerson M, Limpaphayom K (2003a) Safety, acceptability, and feasibility of a single-visit approach to cervical-cancer prevention in rural Thailand: a demonstration project. *Lancet*361(9360): 814–820
15. Gaffikin L, Lauterbach M, Blumenthal PD (2003b) Performance of visual inspection with acetic acid for cervical cancer screening: a qualitative summary of evidence to date. *Obstet Gynecol Surv* 58(8): 543–550
16. Goldie SJ, Gaffikin L, Goldhaber-Fiebert JD, Gordillo-Tobar A, Levin C, Mahe C, Wright TC (2005) Cost-effectiveness of cervical-cancer screening in five developing countries. *N Engl J Med* 353(20): 2158–2168

17. Sankaranarayanan R (2011) *A Practical Manual on Visual Screening for Cervical Neoplasia. [2003]*. IARC Press: Lyon
18. Ngoma T, Muwonge R, Mwaiselage J, Kawegere J, Bukori P, Sankaranarayanan R (2010) Evaluation of cervical visual inspection screening in Dar es Salaam, Tanzania. *Int J Gynaecol Obstet* 109(2): 100–104
19. Nessa A, Hussain MA, Rahman JN, Rashid MH, Muwonge R, Sankaranarayanan R (2010) Screening for cervical neoplasia in Bangladesh using visual inspection with acetic acid. *Int J Gynaecol Obstet* 111(2): 115–118
20. Muwonge R, Manuel MG, Filipe AP, Dumas JB, Frank MR, Sankaranarayanan R (2010) Visual screening for early detection of cervical neoplasia in Angola. *Int J Gynaecol Obstet* 111(1): 68–72
21. Sankaranarayanan R, Esmy PO, Rajkumar R, Muwonge R, Swaminathan R, Shanthakumari S, Fayette JM, Cherian J (2007) Effect of visual screening on cervical cancer incidence and mortality in Tamil Nadu, India: a cluster-randomised trial. *Lancet* 370(9585): 398–406
22. Alliance for Cervical Cancer PreventionACCP. Effectiveness, safety, and acceptability of cryotherapy. A systematic literature review. *Cervical cancer prevention issues in depth, no 10* (2003)
23. Sankaranarayanan R, Wesley R, Somanathan T, Dhakad N, Shyamalakumary B, Amma NS, Parkin DM, Nair MK (1998) Visual inspection of the uterine cervix after the application of acetic acid in the detection of cervical carcinoma and its precursors. *Cancer* 83(10): 2150–2156
24. Sankaranarayanan R, Wesley R, Thara S, Dhakad N, Chandralekha B, Sebastian P, Chithrathara K, Parkin DM, Nair MK (2003) Test characteristics of visual inspection with 4% acetic acid (VIA) and Lugol's iodine (VILI) in cervical cancer screening in Kerala, India. *Int J Cancer* 106(3): 404–408

Chapter 5

Human papillomavirus clearance and persistence after cryotherapy

J.N.I. Vet, G. Purwoto, L. Nuranna, K.H. Nuryanto, G.J. Fleuren, V.T.H.B.M. Smit, J.B. Beltman

Submitted

Abstract

Objective: Cryotherapy is widely used in single visit "see and treat" screening programs for cervical cancer. We investigated type specific HPV clearance after treatment with cryotherapy in a population-based cervical cancer screening program in Indonesia, with special interest for types associated with glandular cell infection.

Methods: HPV testing was performed before and 6 months after cryotherapy in 367 women. Samples were tested for HPV presence and type by PCR and INNO-line probe assay.

Results: Sixty-six (18%) of the samples were HPV positive. The type specific clearance rate 6 months after cryotherapy was 80.3%. The most prevalent persistent high-risk types were 18, 51, and 52. There was no difference in risk of persistence of high-risk HPV types more associated with glandular cells, compared to those more associated with squamous cells, (relative risk 0.63, 95% confidence interval 0.12–3.29). The risk of persistence of low-risk HPV types was significantly higher compared to that of high-risk types (relative risk 3.3, 95% confidence interval 1.66–6.67).

Conclusion: The HPV clearance rate after cryotherapy was high and cryotherapy seems to effectively eradicate HPV, including types associated with glandular cells. The risk of persistence of low-risk HPV types after cryotherapy treatment is significantly higher than for high-risk types.

Keywords: Cryotherapy, HPV, HPV clearance, HPV persistence, See and Treat, Low- and Middle-income countries, pap smear, VIA

Introduction

Cervical cancer is the fourth leading cancer in women in incidence and mortality worldwide with over 600.000 new cases and over 341.000 deaths estimated in 2020, of which approximately 85% of the cases occurred in low-and middle-income countries (LIMC)(1, 2). In Indonesia cervical cancer is even the most common cancer in women, accounting for 25% of all female cancer cases reported from 2008-2012 based on Hospital Based Cancer Registry data (3, 4).

WHO recommendations for See and Treat screening programs for cervical cancer in LMIC countries include treatment of premalignant lesions with cryotherapy if the lesions are eligible (5). Cryotherapy is suitable for low-resource settings, because the treatment is easy to use, does not require electricity, is safe and cost effective. Direct treatment of identified premalignant lesions increases the effectiveness of screening programs, as it minimizes the loss of patients due to inadequate follow-up (5). It has proven to be effective with histology proven cure rates of 94.0% for CIN 1, 92.0% for CIN 2, and 85.0% for CIN 3, and complications and adverse effects are rare (6-8).

The persistence of a human papillomavirus infection (HPV) in the uterine cervix has been established as the cause of cervical cancer (9, 10). Screening with HPV testing and treatment with cryotherapy has been described to lead to a 73% reduced cumulative risk of CIN 2+ lesions 36 months after treatment with cryotherapy compared to a control arm in which further evaluation or treatment was delayed for 6 months (11). And a single round of HPV testing and treatment is associated with a significant reduction in numbers of advanced cervical cancers and associated deaths (HR .52 95% CI 0.33-0.83) (12).

Cryotherapy is an effective method for eradicating HPV infections, and treating premalignant lesions, but a percentage of HPV infections will be persistent after treatment (13). HPV types that infect the mucosal epithelium of the human genital tract are subdivided into various genera. HPV types 16, 31, 33, 35, 52, and 58 are phylogenetically related and grouped as clade a-9, while HPV types 18, 39, 45, 59, and 68 are phylogenetically related and grouped as clade a-7. The clades show differences in preferences with respect to the site of infection. The clade a-9 genotypes are more associated with squamous cell carcinomas (SCC), the clade a-7 genotypes are more commonly associated with adenocarcinomas (ADC) (14, 15). Although most HPV types in these clades can lead to 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 (16, 17). As some HPV types are more associated with squamous cell tissue and others are more associated with glandular cell tissue, there might be a difference in persistence of HPV after cryotherapy due to the location of the lesion. The

cryotherapy probe has been designed to effectively treat both ectocervical and endocervical lesions, but endocervical lesions higher up in the cervical canal might not be reached.

Data from a population-based single-visit screening program for cervical cancer in low resource settings in Indonesia were used (18) for this study. Research on HPV prevalence in this population showed that in Indonesia, the overall HPV prevalence was 11.4% and HPV types 52, 16, and 18 are mostly prevalent (15). In this study, we investigated the effectiveness of cryotherapy on the eradication of HPV infections, when treating premalignant lesions.

Materials and Methods

The approval for this study was given by the institutional review boards of the three collaborating University Hospitals (Cipto Mungunkusumo in Jakarta, Hasan Sadikin in Bandung, Sanglah Bali) and the regional hospital in Tasikmalaya.

Sample collection

Data from an earlier population based single visit cervical cancer screening program conducted in low resource areas in Jakarta, Tasikmalaya (Java) and on the island of Bali were used for this study, this has been described in detail earlier (14). In short, the aim of the project was to screen women in low resource areas with limited access to health care facilities by visiting them in their villages with mobile clinics. The teams consisted of two doctors, a cytologist, two nurses, two public health nurses and a driver, and the women were informed about the project by the 'Pembinaan Kesejahteraan Keluarga' (PKK), the national Indonesian family welfare organization. that has access to the smallest villages. After haven given informed consent, women were submitted to screening using visual inspection with acetic acid (VIA) and Papanicolaou test (Pap smear). For research purposes, a cell sample for HPV detection was collected from each participant. The Pap smear was examined by a trained cytologist on the spot, and were classified according to the Bethesda classification(19). In case of a positive VIA, a biopsy was taken of the lesion and examined by a pathologist in the hospital.
In case the VIA and/or cytology results were positive, cryotherapy was performed. Cryotherapy was provided in cycles of 3 minutes of freeze, 3 minutes of thaw, and 3 minutes of freeze with an Erbe cryogun (Germany) with a cone-shaped probe and CO2 (Jakarta and Tasikmalaya) or N2O (Bali). Women were not eligible for cryotherapy if the lesion was suspected of cervical cancer, extended over more than 75% of the cervix, extended into the cervical canal or vaginal wall, or could not be covered fully by the cryotherapy probe.

All of the women who were treated, were registered for follow-up after 6 months, which consisted of repeating cytology, VIA, and HPV sampling. Women were excluded from the screening programme if they had never been sexually active, were pregnant, had a history of treatment to the cervix, or had a history of cervical cancer.

Women from the screenings programme described above were included in this study investigating HPV persistence after cryotherapy, if they were screened in the screenings programme, treated with cryotherapy and if HPV results were available both prior to and 6 months after cryotherapy was performed.

Classification of results

For this study lesions were classified as high risk when histology results were ≥ CIN II or cytology results were ≥ HSIL, and classified as low risk when histology results were ≤ CIN II or cytology results were ≤HSIL. VIA results were classified negative, positive, or suspicious of cervical cancer.

Typing and analysis of HPV samples

Exfoliated cells remaining on the Pap-smear spatula and brush were suspended in 25 ml of phosphate-buffered saline in a 50 ml Falcon tube. The tubes were centrifuged at 3000 × g for 5 minutes. The supernatant was removed, and the cell pellet was resuspended in 1 ml of phosphate-buffered saline and transferred to a 1.5 ml Eppendorf tube with a safety lock. All of the tubes were directly frozen and stored in a −20°C freezer and shipped on dry ice to the Department of Pathology, Leiden University Medical Center, the Netherlands. To test the quality of DNA obtained from the exfoliated cells, PCR was performed with the human genomic •-globin gene as the control template. HPV DNA was amplified using the SPF10 primer set, and the presence of HPV amplimers was detected on agarose gels. Genotyping of positive products was performed with the INNO-LiPA line-probe assay (Innogenetics). This assay can detect the following 25 HPV types: 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, and 74.

Data management and statistics

Data management was performed with Microsoft Access 2003. Statistical analysis was performed with SPSS software (SPSS, version 12, SPSS Inc., Chicago, IL). To compare proportions, we used chi-square and Fisher's exact tests.

Results

In total 367 women were included in this study. The mean age was 38.2 years (range 18–66 years). Reasons for cryotherapy at time of screening were: positive VIA (44.7%) test, abnormal cytology (6.0%) , and positive results for both tests 181 (49.3%) . Cryotherapy was administered to 315 women with CO2 (Jakarta and Tasikmalaya) and 52 women with N2O (Bali).

Before cryotherapy, 66 out of 367 samples (18.0%) were HPV positive; of these, 57 were single HPV infections and 9 were multiple HPV infections (8x 2 types, 1x 3 types). In total, 76 HPV infections were identified and genotyped; 60 were high risk, 12 were low risk types. In four samples, HPV was detected but could not be classified into the known types; these were referred to as "type X." The most prevalent high-risk HPV types were 52 (n = 15), 53 (n = 9), 18 (n = 8), and 16 (n = 7). See table 1 contains details HPV types.

Of all infections, 29 HPV infections were identified in high-risk lesions, and 33 infections were identified in low-risk lesions. There were 10 HPV infections identified in lesions with a positive VIA test but turned out to have normal histology and normal Pap smears. Another 4 HPV infections were found in lesions with a positive VIA test, normal Pap smear but with histology results indicating infection. See table 2 for details regarding the distribution of HPV types and lesions.

At the follow-up screening 6 months after cryotherapy, the clearance rate in the HPV positive patients was 80.3%; 61 of the 76 detected HPV infections were cleared, 15 infections (19.7%) persisted. In this group also 11 new HPV infections were detected. In the group of 301 samples that were HPV negative before cryotherapy, 25 samples (8.3%) were positive after 6 months; in these samples, the most prevalent HPV types were 54 (n = 7), 74 (n = 4), and 16 (n = 4) (Table 1).

Table 1. HPV types before and 6 months after cryotherapy.

HPV Before Cryotherapy		*n*	Cleared	Persistent	New	HPV after Cryotherapy
Multiple infections						
High risk	16/53	2	2	0		
	18/6	1	1	0		
	18/68	1	0	1		18
	18/70	1	0	1		18/70/45*/68*
	42/43	1	0	1		43
	53/68/74	1	0	1		74
	54/39	1	1	0		
	66/6	1	1	0		
	16/33	1			1	
	31/74	1			1	
	39/52/54/11	1			1	
Low risk	54/74	1			1	
Single infections						
High risk	16	5	4	1	3	16
	18	5	5	0	1	
				1		54*/74*
	31	2	2	0		
	45	1	1	0		
	51	6	4	1		51/53*
				1		51/54*/74*
				1		X*
	52	15	13	2	1	52
				1		54*/66*
	53	6	6	0	1	
	56	1	0	1		56
	58	1	1	0		
	66	4	4	0		
Low risk	6	1	1	1	1	6
	11	1	0	1		11
	44	1	0	1		44
	54	1	1	0	1	
	62	1	0	0		X*
	70	1	1	0	1	
	74				1	
	81	1	0	1	1	81
Unknown	X	4	4	0		

The risk of persistence of low-risk HPV types (6 out of 12 infections, 50.0%) was significantly higher compared to the high-risk HPV types (9 out of 60, 15.0%) (relative risk [RR] 3.3, 95% confidence interval (1.66–6.67). All HPV X types were cleared.

Of the most common high-risk HPV types 52, 53, 18, and 16; respectively, 13.3% (2/15), 0.0% (0/9), 25.0% (2/8), and 14.3% (1/7) persisted 6 months after cryotherapy. The groups were too small to allow us to calculate differences in the risk of persistence among the different HPV types.

Table 2. HPV distribution and persistence according to premalignant lesions

	Before cryotherapy		After cryotherapy	
			Persistent HPV(%)	HPV type
High grade lesions[1]	HR-HPV	27	5 (18.5)	16/18/18/52/70
	LR-HPV	2	1 (50.0)	11
total		29	6 (20.7)	
Low grade lesions[2]	HR-HPV	24	2 (8.3)	51/52
	LR-HPV	7	3 (42.8)	43/44/74
	HPV X	2	0	
total		33	5 (15.2)	
Histology negative	HR-HPV	7	2 (28.6)	51/56
	LR-HPV	3	2 (66.7)	6/81
total		10	4 (40.0)	
VIA + cyt - Histology?	HR-HPV	2	0	
	LR-HPV	0	0	
	HPV X	2	0	
total		4	0	

There was no significant difference in the persistence of clade a-9 HPV types (more associated with squamous cell tissue) compared to the clade a-7 HPV types (more associated with glandular cell tissue): 3/26 (11.5%) for clade a-9 and 2/11 (18.2%) for clade a-7 (Fisher's exact test p = 0.6; RR 0.63, 95% CI 0.12–3.29).

There were no differences in the risk of persistence between the following groups: patients treated with CO2 and those treated with N20, VIA-negative and VIA-positive groups, cytology-negative and cytology-positive groups, cytology-positive and VIA-positive groups, and single-infection and multiple-infection groups, for statistic details see table 3.

Of the 29 HPV infections found in high grade lesions, 6 HPV infections (20.7%) persisted (5x HR-HPV, 1x LR-HPV). Of the 33 HPV infections found in low grade lesions, 5 infections (15.1%) persisted (2xHR-HPV, 3x LR-HPV). Of the 10 infections in the VIA positive, histology negative group, 4 HPV infections (40%) persisted (2xHR-

HPV, 2xLR-HPV). In the VIA positive, histology negative, cytology negative group (4 infections), nil HPV types persisted, for details see table 2.

The overall cure rate after cryotherapy in the 66 women in whom these 76 HPV infections were identified, was 85.7%; 54 women had a negative pap smear and VIA test in follow up, 2 still had a positive VIA, 5 still had positive cytology and 2 had both a positive VIA and Pap smear, for 1 woman the results were incomplete, for 2 the results were missing.

In the 9 women with persisting disease after cryotherapy, HPV 52 was found in one follow up HSIL pap smear, HPV X and HPV 11 were found in two follow up LSIL pap smears, and HPV 18 was found in one follow up VIA positive patient. No HPV was identified in the samples of the other 6 patients with 4 LSIL, 1 ASCUS follow up Pap smears and one positive VIA test.

Table 3. Risk of persistence

Variable	A	B	Risk of persistence
HPV	Low risk-HPV (6/12, 50%)	High risk-HPV (9/60, 15%)	RR 3.3, 95% CI 1.66-6.67
Clade	Clade a-9 (3/26, 11.5%)	Clade a-7 (2/11, 18.2%)	RR 0.63, 95% CI 0.12-3.29
Cryotherapy	CO2 (11/65, 16.9%)	NO2 (4/11, 36.4%)	RR 0.47, 95%CI 0.18-1.20
VIA	VIA negative (4/16, 25%)	VIA positive (11/60, 18.3%)	RR1.36, 95% CI 0.50-3.72
Cytology	Cytology negative (5/26, 19.2%)	Cytology positive (10/50, 20%)	RR 0.96, 95% CI 0.36-2.52
VIA	Cytology positive (10/50, 20%)	VIA positive (11/60, 18.3%)	RR 0.92, 95% CI 0.42-1.98
HPV infections	Single infections (10/55, 18.2%)	Multiple infections (4/9, 44.4%)	RR 0.98, 95% CI 0.60-1.57

Discussion

In this study, we investigated HPV persistence in women treated with cryotherapy in a single-visit screening program for cervical cancer in a rural Indonesian setting. We found that the overall HPV infection clearance rate was 80.3% at the follow-up screening 6 months after cryotherapy, which means a persistence rate of 19.7%. In our study, from the persisting HPV types, the low-risk HPV types were significantly more persistent than high risk HPV types and we did not find any difference in persistence of HPV types more associated with the endocervical glandular cells, the HPV clade-a7.

According to a systematic review of patterns of HPV persistence by Hoffman et al, the median HPV persistence after any kind of cervical treatment tends to decrease with increasing follow up time, from 27% at 3 months after treatment, 21% at 6 months, 15% at 12 months and 10% after 24 months (13). The same pattern of ongoing gradual clearance has been observed after treatment with cryotherapy. In a study by Aerssens et al. the type specific HPV clearance rate of proven CIN 1 lesion was 62.4% at 6 months after cryotherapy and had progressed to 82.3% 24 months after cryotherapy (20). Another study by Elfgren et al showed a clearance rate of 64% at 6 months and 76% at 12 months after treating CIN I and II lesions with cryotherapy (21). Our study showed a 80.3% type specific HPV clearance rate at 6 months. The initial rates of the first studies are lower at 6 months compared to our study, this might be due to the a single-freeze technique they used in cryotherapy, compared to the double-freeze technique used in this study (22). Moreover, their rate of proven pathology was higher than in our population, which might have influenced the clearance. Most of the HPV infections are cleared within 2 years, the 10% that persists longer are highly associated with premalignant lesions. Although many HPV infections are cleared spontaneously within 2 years (23), we can conclude that cryotherapy does effectively increase the clearance rate.

The risk of persistence for low-risk HPV types (50.0%) was significantly higher compared to high-risk HPV types (15.0%) (RR 3.3, 95%CI 1.66–6.67). In two studies by Castle et al, carcinogenic HPV types appeared to have a similar affinity for vaginal and cervical epithelium, but noncarcinogenic HPV types may have a tropism for vaginal epithelium (24, 25). They described this for the α-3, α-4, and α-15 clades (HPV types 2, 27, 57, 61,71, 72, 81, 83, and 84), which were the prevalent HPV types in their study. The same phenomenon was observed for the prevalent noncarcinogenic HPV types in our study, which were largely from clade α-10 (HPV types 6, 11, 13, 44, and 74). A possible explanation for the observed higher risk of persistence of low-risk HPV types is that after destroying the squamous columnar junction (SCJ) with cryotherapy, relatively more low-risk HPV types remain in the vagina and can be detected. The same patterns were observed in two studies after treatment with LEEP, performed by

Lidroth et al, and by Kreimer et al. Their persistency rates were respectively 14% and 18 % for high-risk HPV types compared to 34% and 41% for the low-risk HPV types (26, 27).

The groups were too small to calculate differences in the risk of persistence among the different HPV types. In our study, of the most common high-risk HPV types, HPV 52, 53, 18 and 16, respectively 13.3% (2/15), 0% (0/9), 25.0% (2/8), and 14.3% (1/7) persisted 6 months after cryotherapy. HPV 51 persisted in 33.3% (2/6) of the cases. Data on specific HPV persistence after cryotherapy are limited, in Elgrens study HPV 16 and HPV 18 or a combination were most likely to persist after cryotherapy (21). Women with a persistent high risk HPV infection after treatment have a higher risk for residual/ recurrent disease and require close surveillance (28). Women with a compromised immune status like HIV seropositive women, are at a higher risk for HPV infections to persist and to progress to cervical lesions (29, 30). In addition, lesions in HIV positive women are more likely to recur after treatment therefore it would be important to know the HIV status (31, 32). At the time of this study HIV testing was not feasible due to resistance of the population because of the sensitivity of the subject.

In our study, there was no difference in the risk of persistence after cryotherapy between the HPV types that are associated with glandular cells (clade a-7) compared to those associated with squamous cells (clade a-9) (Fisher's exact test p = 0.6; RR 0.63, 95% CI 0.12–3.29). In an American HPV persistence study using LEEP as treatment modality, there was no difference in rates of persistence between clade a-9 (HPV 16 and related types) and clade a-7 (HPV 18 and related types) 17.6% and 17.9% respectively (27). In cryotherapy the concern of persistence of clade a-7 HPV types is somewhat higher because of the characteristics of the cryotherapy device and its ability to reach into the cervical canal. Earlier studies have shown that the effectivity of the cryotherapy device and the different shapes of the tip (flat or shallow curved) do not influence the effectivity of the treatment (33, 34). Although the numbers in our study are small, we do think the risk of undertreatment by cryotherapy of HPV clade a-7 infections is relatively small, Fisher's exact test p = 0.6; RR 0.63, 95% CI 0.12–3.29 (table 3).

Six months after cryotherapy, the rate of newly diagnosed infections was 8.61% (n = 25) in the 301 samples that were HPV negative before cryotherapy. Of the 30 newly detected HPV infections, 40.0% were high-risk types. Cryotherapy is associated with a significant reduction in newly detected high-risk HPV infections. A South African RCT study shows that after cryotherapy women were 55% (95% CI 0.28–0.71) less likely to have a newly detected high-risk HPV infection compared to women in the control group (no treatment) (35). The authors' explanation lies in the destruction of the SCJ on the surface of the cervix and the cascade of immunological

responses that cryotherapy probably induces. Moreover, re-infection might become more difficult after cryotherapy since the new SCJ migrates deeper into the endocervix (33, 34).

Our study has a few limitations. The follow-up was limited to 6 months; we do not have information on HPV infections over a longer period since this research was part of an existing screening program with a set endpoint. The number of samples available before and after cryotherapy was too small to draw definite conclusions on persistence between the different HPV types; investigations with larger numbers of samples are warranted.

In conclusion, among the small percentage of persisting HPV types, the low-risk types are significantly more likely to persist in the genital tract after cryotherapy compared to high-risk types, and there was no difference in the persistence of HPV types more associated with glandular cells compared to those more associated with squamous cells. Overall the HPV clearance rate after cryotherapy was high, and therefore cure rates of cryotherapy are excellent for treating precancerous cervical lesions these settings if patients are eligible.

Acknowledgements

This study was funded by the Female Cancer Foundation, Leiden University Medical Center, the Netherlands (www. femalecancerfoundation.org).

Conflict of interest

There was no conflict of interest in this study for any of the other authors contributed.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018;68(6):394-424. Epub 2018/09/13.
2. observatory Gc. 2020.
3. Gondhowiardjo S, Christina N, Ganapati NPD, Hawariy S, Radityamurti F, Jayalie VF, et al. Five-Year Cancer Epidemiology at the National Referral Hospital: Hospital-Based Cancer Registry Data in Indonesia. JCO global oncology. 2021;7:190-203. Epub 2021/02/05.
4. Aziz MF. Gynecological cancer in Indonesia. Journal of gynecologic oncology. 2009;20(1):8-10. Epub 2009/05/28.
5. Santesso N, Mustafa RA, Schunemann HJ, Arbyn M, Blumenthal PD, Cain J, et al. World Health Organization Guidelines for treatment of cervical intraepithelial neoplasia 2-3 and screen-and-treat strategies to prevent cervical cancer. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2016;132(3):252-8. Epub 2016/02/13.
6. Sauvaget C, Muwonge R, Sankaranarayanan R. Meta-analysis of the effectiveness of cryotherapy in the treatment of cervical intraepithelial neoplasia. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2013;120(3):218-23. Epub 2012/12/26.
7. Martin-Hirsch PP, Paraskevaidis E, Bryant A, Dickinson HO. Surgery for cervical intraepithelial neoplasia. The Cochrane database of systematic reviews. 2013(12):Cd001318. Epub 2013/12/05.
8. Basu P, Taghavi K, Hu SY, Mogri S, Joshi S. Management of cervical premalignant lesions. Current problems in cancer. 2018;42(2):129-36. Epub 2018/02/13.
9. zur Hausen H. Human papillomaviruses in the pathogenesis of anogenital cancer. Virology. 1991;184(1):9-13. Epub 1991/09/01.
10. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. The Journal of pathology. 1999;189(1):12-9. Epub 1999/08/19.
11. Denny L, Kuhn L, Hu CC, Tsai WY, Wright TC, Jr. Human papillomavirus-based cervical cancer prevention: long-term results of a randomized screening trial. Journal of the National Cancer Institute. 2010;102(20):1557-67. Epub 2010/10/05.
12. Sankaranarayanan R, Nene BM, Shastri SS, Jayant K, Muwonge R, Budukh AM, et al. HPV screening for cervical cancer in rural India. The New England journal of medicine. 2009;360(14):1385-94. Epub 2009/04/03.
13. Hoffman SR, Le T, Lockhart A, Sanusi A, Dal Santo L, Davis M, et al. Patterns of persistent HPV infection after treatment for cervical intraepithelial neoplasia (CIN): A systematic review. Int J Cancer. 2017;141(1):8-23. Epub 2017/01/27.
14. Bernard HU, Burk RD, Chen Z, van Doorslaer K, zur Hausen H, de Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology. 2010;401(1):70-9. Epub 2010/03/09.
15. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. Virology. 2004;324(1):17-27. Epub 2004/06/09.
16. Seoud M, Tjalma WA, Ronsse V. Cervical adenocarcinoma: moving towards better prevention. Vaccine. 2011;29(49):9148-58. Epub 2011/10/11.

17. Clifford G, Franceschi S. Members of the human papillomavirus type 18 family (alpha-7 species) share a common association with adenocarcinoma of the cervix. Int J Cancer. 2008;122(7):1684-5. Epub 2007/12/07.
18. Vet JN, Kooijman JL, Henderson FC, Aziz FM, Purwoto G, Susanto H, et al. Single-visit approach of cervical cancer screening: see and treat in Indonesia. British journal of cancer. 2012;107(5):772-7. Epub 2012/08/02.
19. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. Jama. 2002;287(16):2114-9. Epub 2002/04/23.
20. Aerssens A, Claeys P, Garcia A, Sturtewagen Y, Velasquez R, Vanden Broeck D, et al. Natural history and clearance of HPV after treatment of precancerous cervical lesions. Histopathology. 2008;52(3):381-6. Epub 2008/02/14.
21. Elfgren K, Jacobs M, Walboomers JM, Meijer CJ, Dillner J. Rate of human papillomavirus clearance after treatment of cervical intraepithelial neoplasia. Obstetrics and gynecology. 2002;100(5 Pt 1):965-71. Epub 2002/11/09.
22. Schantz A, Thormann L. Cryosurgery for dysplasia of the uterine ectocervix. A randomized study of the efficacy of the single- and double-freeze techniques. Acta obstetricia et gynecologica Scandinavica. 1984;63(5):417-20. Epub 1984/01/01.
23. de Sanjose S, Brotons M, Pavon MA. The natural history of human papillomavirus infection. Best practice & research Clinical obstetrics & gynaecology. 2018;47:2-13. Epub 2017/10/02.
24. Castle PE, Schiffman M, Bratti MC, Hildesheim A, Herrero R, Hutchinson ML, et al. A population-based study of vaginal human papillomavirus infection in hysterectomized women. The Journal of infectious diseases. 2004;190(3):458-67. Epub 2004/07/10.
25. Castle PE, Rodriguez AC, Porras C, Herrero R, Schiffman M, Gonzalez P, et al. A comparison of cervical and vaginal human papillomavirus. Sexually transmitted diseases. 2007;34(11):849-55. Epub 2007/07/11.
26. Lindroth Y, Bjelkenkrantz K, Forslund O. Spectrum of HPV types before and after treatment of cervical intraepithelial neoplasia grade 2 and 3. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2017;97:38-43. Epub 2017/11/04.
27. Kreimer AR, Katki HA, Schiffman M, Wheeler CM, Castle PE. Viral Determinants of Human Papillomavirus Persistence following Loop Electrical Excision Procedure Treatment for Cervical Intraepithelial Neoplasia Grade 2 or 3. Cancer Epidemiology Biomarkers & Prevention. 2007;16(1):11-6.
28. Mariani L, Sandri MT, Preti M, Origoni M, Costa S, Cristoforoni P, et al. HPV-Testing in Follow-up of Patients Treated for CIN2+ Lesions. Journal of Cancer. 2016;7(1):107-14. Epub 2016/01/02.
29. Ahdieh L, Klein RS, Burk R, Cu-Uvin S, Schuman P, Duerr A, et al. Prevalence, incidence, and type-specific persistence of human papillomavirus in human immunodeficiency virus (HIV)-positive and HIV-negative women. The Journal of infectious diseases. 2001;184(6):682-90. Epub 2001/08/23.
30. Denslow SA, Rositch AF, Firnhaber C, Ting J, Smith JS. Incidence and progression of cervical lesions in women with HIV: a systematic global review. International journal of STD & AIDS. 2014;25(3):163-77. Epub 2013/11/13.

31. De Vuyst H, Mugo NR, Franceschi S, McKenzie K, Tenet V, Njoroge J, et al. Residual disease and HPV persistence after cryotherapy for cervical intraepithelial neoplasia grade 2/3 in HIV-positive women in Kenya. PloS one. 2014;9(10):e111037. Epub 2014/10/25.
32. Oga EA, Brown JP, Brown C, Dareng E, Adekanmbi V, Odutola M, et al. Recurrence of cervical intraepithelial lesions after thermo-coagulation in HIV-positive and HIV-negative Nigerian women. BMC women's health. 2016;16:25. Epub 2016/05/14.
33. Stienstra KA, Brewer B, Franklin LA. A Comparison of Flat and Shallow Conical Cervical Cryotherapy Tips: Elimination of CIN and Posttreatment Location of the Squamocolumnar Junction. Journal of lower genital tract disease. 1999;3(1):46. Epub 1999/01/01.
34. Stienstra KA, Brewer BE, Franklin LA. A comparison of flat and shallow conical tips for cervical cryotherapy. The Journal of the American Board of Family Practice. 1999;12(5):360-6. Epub 1999/10/26.
35. Taylor S, Wang C, Wright TC, Denny L, Tsai WY, Kuhn L. Reduced acquisition and reactivation of human papillomavirus infections among older women treated with cryotherapy: results from a randomized trial in South Africa. BMC medicine. 2010;8:40. Epub 2010/07/01.

Chapter 6

A performance evaluation of an optoelectronic cervical screening device in comparison to cytology and HPV DNA testing

Jessica N Vet, James P. Haindl, Carolina Velasquez, Leonie J. Parker, Margaret I. Burns, Stephen Morrell, Michael J. Campion

Adapted from European Journal of Gynaecological oncology Vol. 43 Issue 2, April 2022 pp. 213-218

Abstract

Objective: An optoelectronic screening device (OESD) is evaluated for the detection of cervical intra-epithelial neoplasia (CIN) 2+ lesions in comparison to Liquid Based Cytology (LBC) and high-risk HPV DNA (hrHPV) testing.

Methods: In total 506 consecutive women referred because of abnormal cervical cytology or a positive high-risk HPV test, had an examination using OESD, LBC, and hrHPV testing. They were screened in 4 colposcopy clinics in New South Wales, Australia. In a retrospective audit, results were compared to the gold standard of colposcopy and biopsies if required. Sensitivity, specificity, area under the receiver operating characteristic (ROC) curves, and differences using McNemar tests were calculated. All results were available for comparison on 474 patients.

Results: The sensitivity to detect CIN II+ lesions by OESD, LBC and hrHPV-testing was 0.72, 0.81, and 0.88, and the specificity was 0.71, 0.95, and 0.76 respectively. The age- and previous-treatment adjusted area under the ROC curve for OESD was 0.83, for LBC 0.93, and for hrHPV testing 0.88. McNemar's tests showed no significant difference in sensitivity between OESD and LBC (p value = 0.26), and no significant difference in specificity between OESD and hrHPV-testing (p = 1.0) amongst patients without previous treatment.

Conclusion: The optoelectronic screening device demonstrated comparable sensitivity to high quality cytology conducted in a hospital clinical setting. Specificity was comparable to hrHPV-testing in an approximate primary screening setting. OESD has the advantage of producing an immediate result and being easy to use without need of laboratory equipment. This device can potentially become an important tool in the prevention of cervical cancer, particularly in developing countries and resource-limited settings.

1. Introduction

Cervical cancer is the third most common cancer in females worldwide, with an estimated 570,000 new cancer cases annually. In developing countries, where more than 85% of the new cervical cancer cases occur, it is the second most common cancer among women after breast cancer (1-4). A persistent infection with high-risk human papillomavirus (hrHPV) in the uterine cervix has been established as the primary cause of cervical cancer (5-7). Cervical cancer has detectable premalignant stages, which offer major opportunities for screening, early treatment of pre-cancer and cancer and a consequent reduction in cancer incidence and mortality (8,9). In developed countries, introduction of organised exfoliative cytology-based screening programs have led to marked reductions in the incidence of cervical cancer (10-12). In addition, improved screening programs using hrHPV testing and the introduction of HPV vaccination programmes leading to reduced incidence of high-grade precancerous cervical lesions suggest that cervical cancer incidence rates will fall even further (13).

In low- and middle-income countries (LMI), successful implementation of organised cervical screening often fails due to lack of financial support and properly trained cytologists, and poor laboratory and program support services (14-15). Until a prophylactic HPV vaccine covering all hrHPV types becomes available, there will continue to be a need to screen and treat women for cervical premalignant lesions. Cervical screening alternatives which are simpler to implement, acceptable to women and cost-effective are still being researched. One of these alternatives is a real-time optoelectronic device. These devices are handheld and use electrical and optical signals to classify cervical tissue into normal and abnormal. They provide an immediate result without the need for laboratory facilities or qualified cytologists. Earlier studies reported real-time optoelectronic devices to be safe and feasible [(16-18).

In this retrospective audit, we evaluated the performance of the optoelectronic cervical screening device TruScreen as a single screening method to detect cervical intraepithelial neoplasia (CIN 2+). A comparison was made to the performance of liquid-based cytology (LBC) and high-risk HPV DNA testing in the same women in a research setting with colposcopy and histology of colposcopically directed biopsies as the gold standard. Sensitivity and specificity were assessed, and any adverse effects of the optoelectronic screening device were recorded and evaluated.

2. Materials and Methods

From June until December 2017, consecutive women with an abnormal pap smear referred to the colposcopy clinic at the Royal Hospital for Women (RHW) in Sydney, to the Orange Aboriginal Medical Service (OAMS) in Orange, to Tottenham Multipurpose Hospital (TMH) in Tottenham, or to Pius X Aboriginal Medical Service (PXAMS) in Moree, all in New South Wales Australia, consented to be screened with the optoelectronic cervical screening device TruScreen as part of an assessment of its clinical performance. This screening was in addition to the standard hr-HPV and LBC screening, and colposcopic examination.

Women over the age of 18 years, who had agreed to the additional procedure were eligible. Exclusion criteria were current menstrual period, current or recent pregnancy (within 4 months post-delivery), Pap smear within 6 weeks, surgical treatment to the cervix within the past 3 months, previous pelvic radiation, chemotherapy in the previous 5 weeks, clinically apparent acute or subacute cervical infection, photosensitizing disease, and previous hysterectomy.

All women were first screened using the TruScreen Handheld Device, following which a sample was taken for LBC and hrHPV testing. All women then had a colposcopic examination performed by an experienced colposcopist, and abnormal areas were biopsied.

The results for the TruScreen device were immediately available and categorized as: normal (normal squamous epithelium, columnar epithelium, physiologic metaplasia, or latent HPV-related changes) or abnormal (CIN I-III, invasive cancer). All liquid based cytologic and histologic specimens were processed by the department of Anatomical Pathology, South-Eastern Area Laboratory Service (SEAL) in Prince of Wales Hospital, Sydney. LBC samples were classified according to the Australian Modified Bethesda System (19). Results of biopsies were categorized as normal, CIN I, CIN II, CIN III, AIS, and invasive carcinoma (squamous, adenosquamous, adenocarcinoma).

The samples were tested for high-risk HPV DNA using Cobas HPV Assay (Roche Cobas 4800) and were categorized as: HPV negative, HPV 16 positive, HPV 18 positive, HPV positive “other”. The results of the TruScreen test were only known to the team present at the clinical examination. All complications or adverse outcomes were recorded.

2.1 Description of TruScreen

The optoelectronic TruScreen device measures physical properties of tissue. By comparing characteristics of the tissue of interest with the behaviour of known tissue

types, the device can categorise tissue. In this way, it can detect pre-cancerous and cancerous changes in the cervix. Two types of physical measurements are used, optical and electrical.

The device is composed of a hand-held probe coupled with a wireless electromagnetic induction Qi charging cradle. The instrument is approximately 37 cm in length from base to tip (Figs. 1,2). The section of the probe that enters the vagina is 120 mm in length with a tip diameter of approximately 5mm. The system incorporates a single-use sensor (SUS) –encompassed in a sheath which covers the probe of the handpiece, increasing the tip diameter to approximately 6.5 mm.

Fig. 1. TruScreen ultra handheld device.

The tip of the probe interrogates the tissue by repetitively pulsing it with low levels of optical and electrical energy. Real-time interpretation of the cervical tissue response is achieved by automatic comparison with a digitally stored catalogue of tissue signatures. The device measures the directly reflected light, backscattered light and electrical decay curves of the cervical tissue. It assesses the response of surface epithelial cells, but also identifies changes in the epithelial basal layer and stromal cells. These changes include enlarged cell nuclei, increased cytoplasmic density, increased blood circulation and variations in blood vessels, and changes that occur with neoplastic lesions.

The device is powered by a lithium-ion battery with approved patient protection and delivers several electrical pulses of millisecond duration. Twenty-seven pulses are delivered per “observation”, and fourteen observations are made per second. These very low energy pulses are below normal sensation thresholds.

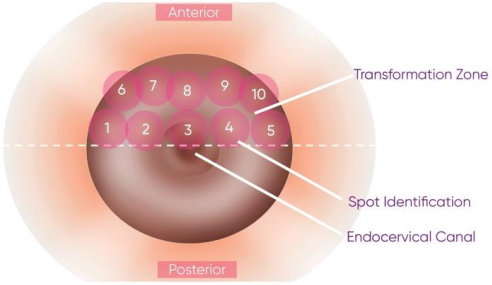


Fig. 2.1 Probing pattern images as depicted in the TruScreen instruction manual. Probing on the outer area of the ectocervix should begin at Spot 1 on the left-hand side and continue in a horizontal direction, working from left to right. Complete two rows, ensuring full coverage of the anterior part of the ectocervix.

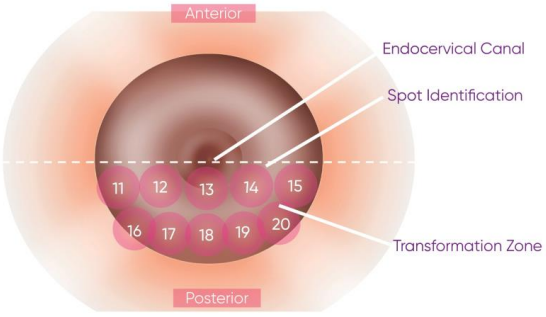


Fig. 2.2 The posterior portion of the ectocervix should proceed in a similar horizontal direction, working from left to right, as shown above.

The optical measurements operate within the visible and near infrared spectrum. The light emitting diodes (LEDs) have a power output range of 20–200 microwatts. The light intensity is far below that of the colposcope. Four LEDs are used to emit light at three discrete wavelengths: green 520 ± 10 nm, near and distant red 660 ± 10 nm, and infra-red 936 ± 15 nm. Per observation, the LEDs operate for approximately one hundredth of a second, and approximately 14 observations are made per second.

The tissue classification algorithm is a pattern-matching expert system. The final output is a result of algorithmic operation on three levels – the observation level, the "spot" level, and the overall patient screening result. With each tissue "observation" approximately 70 optical and electrical parameters are measured.

A "spot" is defined as a series of observations made with the tip of the TruScreen device kept at one location on the cervix. Under prompting from the device LCD screen, the operator moves the tip of the TruScreen probe around the ectocervix, the everted portion of the endocervix and the distal endocervical canal (Fig. 2). To cover the whole squamocolumnar junction a minimum of 15 spots is required for each patient. A maximum number of 32 spot measurements can be taken by the device during each examination. Only after the minimum 15 spot measurements have been performed will the operator be able to conclude the examination and allow for the data to be processed via the patient-level algorithm. The TruScreen device is an expert system and, as for all expert systems, the recognition algorithm had to be "trained". The optical and electrical data were related to an independent "gold standard" reference diagnosis for the training data set. For algorithm training during TruScreen's development, extensive reference data were obtained, including colposcopic and histologic information.

2.2 Statistical analysis

Statistical analysis was performed using SAS software (SAS version 9.4, SAS Inc, USA). Sensitivity and specificity results were calculated, and classification accuracy was quantified by ROC curves for all three screening modalities. McNemar's test was used to derive the associated *p* values for the significance of the differences in sensitivity and specificity.

3. Results

In total, 506 women were recruited. Of these patients, 498 were successfully screened using the optoelectronic device, and no adverse effects occurred. In 8 patients, the optoelectronic examination failed due to rebooting of the device in the first screening attempt. A total of 23 patients were excluded for the following reasons: 9 patients were screened twice, 10 patients were screened within three months of treatment/punch biopsy, 3 patients were screened less than four months postpartum and 1 patient was found to have an acute and severe cervicitis.

Of the final 475 patients, 246 were referred with their first abnormal Pap smear and 229 were having a follow-up colposcopic examination due to previous treatment for a CIN lesion. In total, 393 patients were from the RHW, 44 from PXAMS, 28 from OAMS, and 2 from TMH. The mean age was 37.9, range 19–82 years, standard deviation 11.5 years. For the optoelectronic device and for hrHPV testing, 475 results were available for comparison with the gold standard whilst for LBC, 474 results were available.

Histology of colposcopically-directed biopsies were as follows: HPV changes n=35, CIN I n = 28, CIN II n = 25, CIN III n = 48, micro-invasive squamous cell carcinoma n = 3, and adenocarcinoma in situ n = 1. See Fig. 3 for the flowchart.

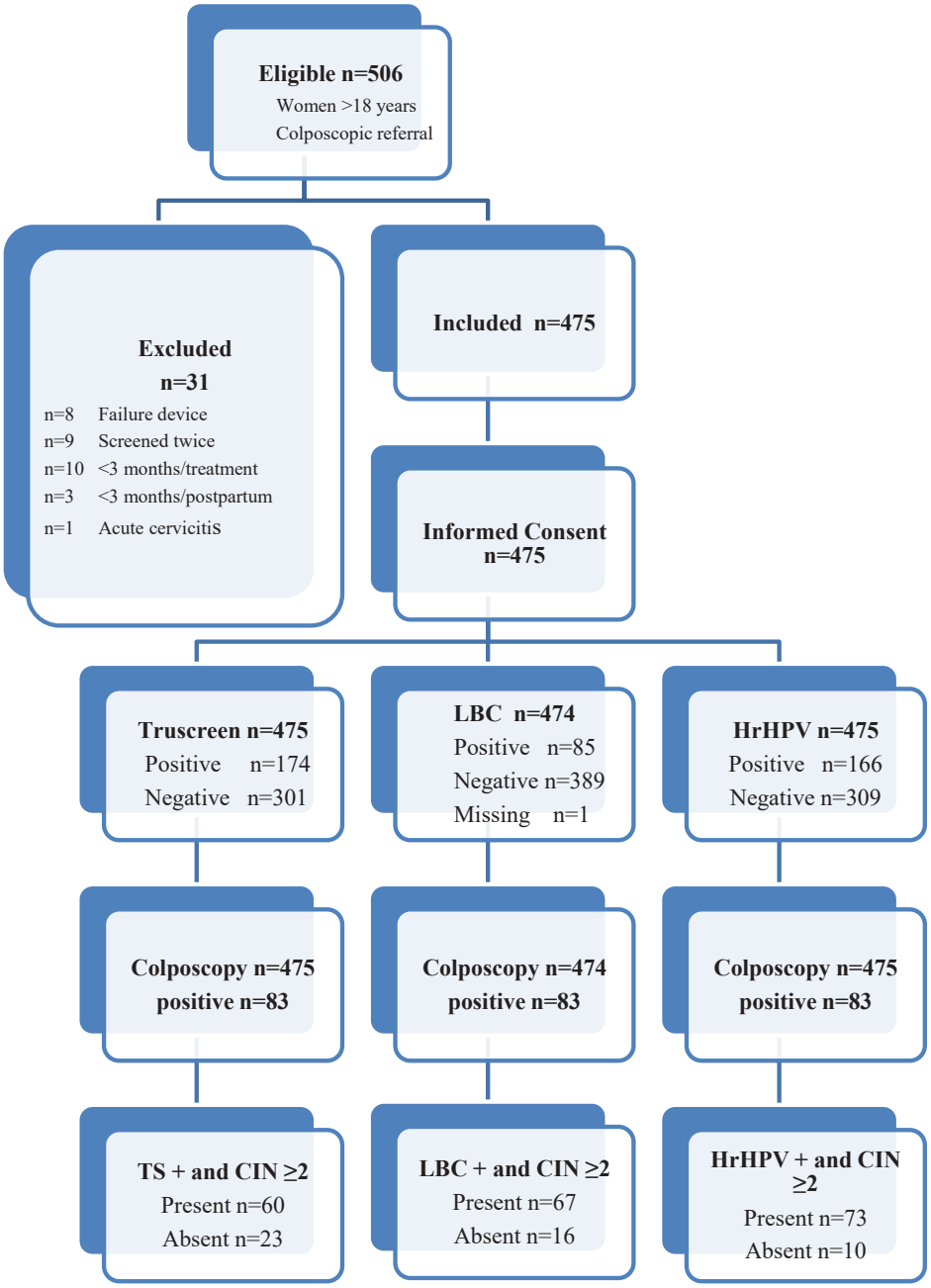


Fig. 3. Flowchart.

3.1 Comparison between the performance of the optoelectronic device, hpv testing, and LBC screening

Using histology of colposcopically directed biopsies as the gold standard, the overall sensitivity for detection of CIN II + lesions for the optoelectronic device, LBC and HPV testing was 0.72, 0.81, and 0.88, respectively; the specificity was 0.71, 0.95, and 0.76, respectively (Table 1).

Results for patients without previous treatment, which more closely approximates primary screening sensitivity, were 0.71 for TruScreen, 0.82 for LBC and 0.88 for HPV, and specificities were 0.72, 0.93, and 0.70, respectively.

For follow up screening, the sensitivity was 0.80 for TruScreen, 0.70 for LBC and 0.90 for HPV testing, and the specificity 0.70, 0.97, and 0.82, respectively.

Location of the screening, the person performing the screening, and the experience of the person performing the screening did not significantly influence the result of the TruScreen test (results not shown).

Table 1. Sensitivity and specificity for the different screening modalities, overall, without previous treatment, after previous treatment.

Test efficacy indicator	**TruScreen**	**LBC**	**HPV**
Overall			
Sensitivity	0.72	0.81	0.88
Specificity	0.71	0.95	0.76
No previous treatment			
Sensitivity	0.71	0.82	0.88
Specificity	0.72	0.93	0.70
Previous treatment			
Sensitivity	0.80	0.70	0.90
Specificity	0.70	0.97	0.82

The unadjusted area under the ROC curves was 0.71 for TruScreen, 0.82 for HPV testing, and 0.88 for LBC; the age-adjusted area under the ROC curves was 0.74, 0.85, and 0.91, respectively. The age- and past treatment adjusted area under the ROC curves was 0.83, 0.89 and 0.94, respectively See Fig. 4 for the age- and past treatment adjusted ROC curves.

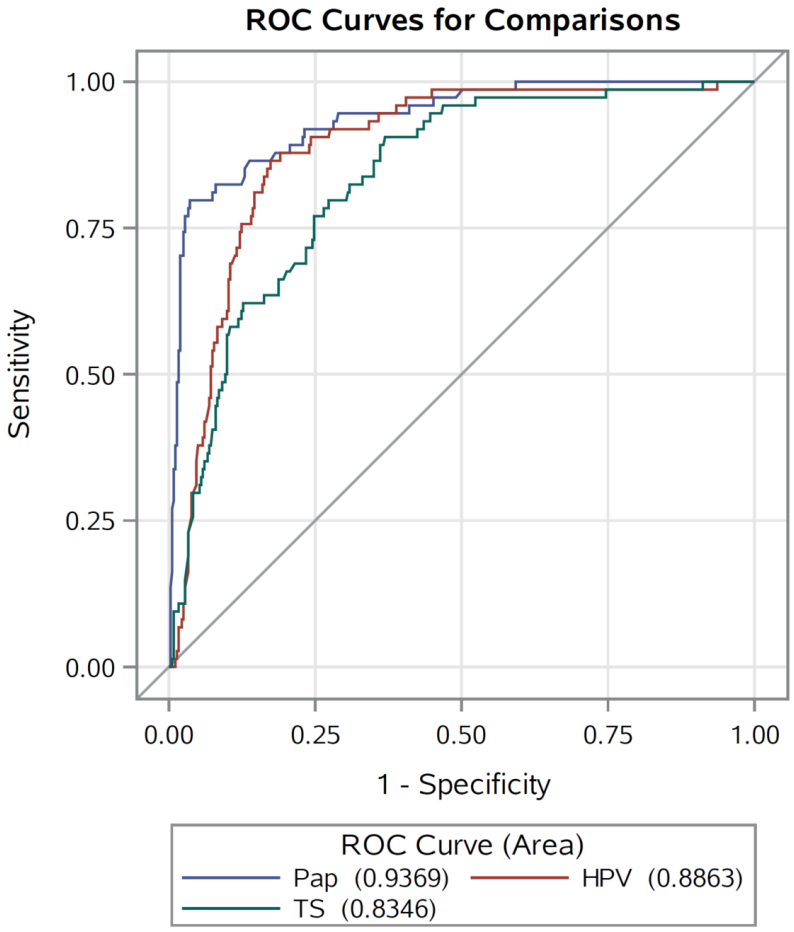


Fig. 4. Age- and past-treatment adjusted combined ROC curves for comparison of the different screening modalities.

McNemar's test of difference did not show a statistically significant difference between the sensitivity for detection of CIN II+ lesions of TruScreen versus LBC, p = 0.26, but did show LBC to be significantly more specific. These results were consistent in the overall group, those without previous treatment, and for those with previous treatment. HPV was more sensitive than TruScreen overall, and in those without previous treatment, and were equally specific in those that hadn't had previous treatment (p = 1.0). For details see Table 3

Table 2. McNemar's test of difference between cervical screening methods (*p* value), overall, without previous treatment, after previous treatment.

Item	TruScreen/LBC	TruScreen/HPV
Overall		
Sensitivity	0.26	0.01
Specificity	<0.001	0.03
No previous treatment		
Sensitivity	0.14	0.02
Specificity	<0.001	1.00
Previous treatment		
Sensitivity	0.65	0.31
Specificity	<0.001	0.004

4. Discussion

In this study, we evaluated the performance of an optoelectronic device, TruScreen, in a research setting for detection of CIN 2+ cervical lesions using the gold standard of histology of colposcopically-directed biopsies. TruScreen was compared to the performance of LBC and high-risk HPV DNA testing. All patients consented to the use of the optoelectronic screening device. The screening was well tolerated, and no adverse effects were reported. Patients responded positively regarding the immediate result.

The sensitivity for detection of CIN 2+ lesions by TruScreen in the overall group was found to be 0.71, which is comparable to sensitivity results described in earlier optoelectronic device studies by Singer *et al* (18) 0.70, and by Lee *et al.* (20) 0.77. A systematic review and meta-analysis of nine Chinese studies, that included 2730 patients with 567 cases of cervical neoplasia, reported a pooled sensitivity of an optoelectronic device (TruScreen) of 0.76 (21). An Indonesian study reported their sensitivity to be 0.76 (22). Both these studies included sensitivities for all CIN lesions and didn't categorise their results into detection of CIN 2+ lesions. Ozgu *et al* and Pruski *et al.* have reported sensitivities as high as 0.86 (23) and 0.90 (24) with the TruScreen optoelectonic device.

The sensitivity of optoelectronic screening might be influenced by failed detection of very small lesions or exclusively endocervical lesions. To increase sensitivity, optoelectronic devices might be used in combination with cytology, as described by Rahmadhany (22) and Singer (18). In these studies, the sensitivity

increased significantly to rates as high as 92.8%. However, combining optoelectronic screening with cytology undermines the major advantages of low cost and the immediate availability of the result.

In our study, the overall specificity for TruScreen was 0.71, comparable to a pooled specificity of 0.69 described by Yang *et al*. (21). The specificity of TruScreen was also comparable to the 0.76 found for hrHPV testing in this study, but both were less than the 0.94 obtained by the liquid-based cytology. The implication for lower specificity is a higher false positive rate and consequently a higher referral rate for further cytology and/or colposcopy + biopsy.

In this study, LBC performed very well. Unfortunately, in many LMIC settings, use of LBC and successful implementation of organised cytology-based cervical cancer screening has failed due to high cost, lack of trained cytologists and poor laboratory and program infrastructure (14,15). In contrast, the sensitivity for the TruScreen optoelectronic device was comparable to the sensitivity obtained by LBC in perfect clinical circumstances. The specificity of TruScreen was comparable to the specificity of hrHPV testing in the group without previous treatment (McNemar's test of difference: $p = 1.0$). This group approximates a primary screening setting. Given these characteristics, this device could potentially become an important tool in a primary screening setting, particularly in developing countries and resource-limited settings.

There is a risk of missing endocervical lesions due to the design of the TruScreen probe which mainly screens the visible cervical surface. In our study, the one endocervical lesion identified by liquid-based cytology, HPV DNA testing and colposcopy, was also identified by TruScreen, presumably because it was low in the canal and accessible to the probe. Future devices would benefit from the development of a probe that could be passed into the canal and emit electrical impulses and light waves horizontally.

A strength of this study is the high-quality setting in which the screening with LBC, hr-HPV testing and the golden standard of colposcopy were performed. Their optimal performance, gives a reliable result when compared to the opto-electronic device. A limitation of the study is that the study was performed in a referred population, which serves the assessment of the performance of the optoelectronic device, but the results should also be confirmed in a population-based setting.

In the search for more objective, reproducible cervical cancer screening, artifical intelligence is being used to classify cervical lesions on images from colposcopy (25). Schiffman *et al* conducted an observational study in which they used an image analyzer that performed "automated visual evaluation" of the cervix as a primary screening method. In their cohort they found excellent sensitivity for detection of CIN 2+ (26). The potential combined AI -TruScreen application lends itself well to the utilization of optoelectronic cervical screening for identifying the location of cervical lesions as an aid to colposcopy (as per Z-Scan). This requires spot-by-spot analysis and reporting of detected abnormalities with an indication of suspected severity. The

only current application of the TruScreen device is screening for cervical neoplasia. To this end, the device gives a “negative” or “positive” result with respect to a screen-detected abnormality, leading to an appropriate clinical response, specifically colposcopy and/or treatment. AI has limited application in this context: while this is a promising way to improve cervical cancer screening, the same limitation in screening of endocervical lesions applies.

5. Conclusions

The optoelectronic device TruScreen demonstrated comparable sensitivity to high quality cytology conducted in a teaching hospital setting, and specificity comparable to hrHPV testing in a setting which approximated primary screening. It has the potential to become an important tool in the prevention of cervical cancer, particularly in developing countries and resource-limited settings, due to the immediate availability of results, the objectivity and non-invasive character of the test and the relative ease of learning the technique. Further studies are required under field conditions to assess its performance and effectiveness in the primary screening setting.

References

[1 Arbyn M, Castellsagué X, de Sanjosé S, Bruni L, Saraiya M, Bray F, et al. Worldwide burden of cervical cancer in 2008. Annals of Oncology. 2011; 22: 2675–2686.
[2] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018 GLOBOCAN estimates of incidence and mortality for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68: 394-424.
[3] Torre LA, Siegel RL, Ward EM, Jemal A. Global Cancer Incidence and Mortality Rates and Trends--An Update. Cancer Epidemiology Biomarkers & Prevention. 2016; 25: 16–27.
[4] Ferlay J, Shin H, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International Journal of Cancer. 2010; 127: 2893–2917.
[5] zur Hausen H. Human papillomaviruses in the pathogenesis of anogenital cancer. Virology. 1991; 184: 9–13.
[6] Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. The Journal of Pathology. 1999; 189: 12–19.
[7] Bosch FX, Lorincz A, Muñoz N, Meijer CJLM, Shah KV. The causal relation between human papillomavirus and cervical cancer. Journal of Clinical Pathology. 2002; 55: 244–265.
[8] Denny L, Kuhn L, Hu C, Tsai W, Wright TC. Human papillomavirus-based cervical cancer prevention: long-term results of a randomized screening trial. Journal of the National Cancer Institute. 2010; 102: 1557–1567.
[9] Sankaranarayanan R. Screening for cancer in low- and middle-income countries. Annals of Global Health. 2014; 80: 412–417.
[10] Lăără E, Day NE, Hakama M. Trends in mortality from cervical cancer in the Nordic countries: association with organised screening programmes. Lancet. 1987; 1: 1247–1249.
[11] Anderson GH, Boyes DA, Benedet JL, Le Riche JC, Matisic JP, Suen KC, et al. Organisation and results of the cervical cytology screening programme in British Columbia, 1955-85. British Medical Journal (Clinical Research Ed.). 1988; 296: 975–978.
[12] Quinn M, Babb P, Jones J, Allen E. Effect of screening on incidence of and mortality from cancer of cervix in England: evaluation based on routinely collected statistics. British Medical Journal (Clinical research ed.). 1999; 318: 904–908.
[13] Cohen PA, Jhingran A, Oaknin A, Denny L. Cervical cancer. Lancet (London, England). 2019; 393: 169–182.
[14] Tsu VD, Levin CE. Making the case for cervical cancer prevention: what about equity? Reproductive Health Matters. 2008; 16: 104–112.
[15] Sankaranarayanan R, Budukh AM, Rajkumar R. Effective screening programmes for cervical cancer in low- and middle-income developing countries. Bulletin of the World Health Organization. 2001; 79: 954–962.
[16] Long S, Lei W, Feng Y, Lv D, Cai Y, Yang P. The feasibilities of TruScreen for primary cervical cancer screening: a self-controlled study. Archives of Gynecology and Obstetrics. 2013; 288: 113–118.
[17] Pruski D, Kedzia W, Przybylski M, Jozefiak A, Kedzia H, Spaczynski M. [Assesment of real optoelectronic method in the detection of cervical intraepithelial neoplasia]. Ginekologia polska. 2008; 79: 342–346.
[18] Singer A, Coppleson M, Canfell K, Skladnev V, Mackellar G, Pisal N, et al. A real time optoelectronic device as an adjunct to the Pap smear for cervical screening: a multicenter evaluation. International Journal of Gynecological Cancer. 2003; 13: 804–811.

[19] Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA. 2002; 287: 2114–2119.
[20] Lee SJ, Bae JH, Kim JH, Lee SH, Namkoong SE, Park JS. A real-time optoelectronic device in screening of cervical intraepithelial meoplasia. Journal of Women's Medicine. 2009; 2: 23–28.
[21] Yang H, Zhang X, Hao Z. The diagnostic accuracy of a real-time optoelectronic device in cervical cancer screening: a PRISMA-compliant systematic review and meta-analysis. Medicine. 2018; 97: e11439.
[22] Rahmadhany R, Indarti J. A real-time optoelectronic device in screening of precancerous cervical lesion. Indones J Obstet Gyncol. 2015; 3: 117–120.
[23] Özgü E, Yıldız Y, Özgü BS, Öz M, Danışman N, Güngör T. Efficacy of a real time optoelectronic device (TruScreen™) in detecting cervical intraepithelial pathologies: a prospective observational study. Journal of the Turkish German Gynecological Association. 2015; 16: 41–44.
[24] Pruski D, Przybylski M, Kedzia W, Kedzia H, Spaczynski M, Jagielska-Pruska J. Optoelectronic method for detection of cervical intraepithelial neoplasia and cervical cancer. Opto-electronics Review. 2011; 19: 478–485.
[25] Shiraz A, Crawford R, Egawa N, Griffin H, Doorbar J. The early detection of cervical cancer. the current and changing landscape of cervical disease detection. Cytopathology. 2020; 31: 258–270.
[26] Hu L, Bell D, Antani S et al. An observational study of deep learning and automated evaluation of cervical images for cancer screening. JNCI J Natl Cancer Inst. 2019; 111 (9): 923-932

Chapter 7

Summary and general discussion

Chapter 7 Summary and general discussion

Cervical cancer is a major public health problem, especially in low- and middle-income countries where the health care infrastructure often prohibits successful implementation of organized screening programs due to lack of financial support, professional human resources, and laboratory services (1-4). Indonesia is a low-income country with high rates of cervical cancer, with limited organized screening programs especially in low resource settings (5, 6).

In this thesis several aspects of cervical cancer in Indonesia have been studied: starting with a description of the prevalent HPV types in Indonesia in a hospital-based population and in a population-based setting (**chapter 2 and 3**). An alternative way of cervical cancer screening in a single visit approach has been set up in low resource areas in Indonesia and is described in **chapter 4**: "See and Treat" using visual inspection with 3-5% acetic acid (VIA) and treatment with cryotherapy. Furthermore, we investigated the HPV types persisting after cryotherapy (**chapter 5**). In the last chapter we assessed the performance of an objective tool for cervical cancer screening; screening with an optoelectronic device **(chapter 6)**. Below, the results obtained, and the main conclusions are discussed and put in perspective for future implications.

Human papilloma Virus (HPV)

HPV 16 and 18 are responsible for 70% of the cervical cancers worldwide, overall HPV 16 causes 50% of the cervical cancers and HPV 18 20% (7-9). HPV prevalence, age-specific prevalence, and type of distribution differ substantially between populations (10-13). This underlines the importance to know the prevalent types of HPV in a country to estimate the impact of future HPV vaccination programmes.

In Indonesia, HPV 18 has a greater role in cervical cancer than in other parts of the world; HPV 18 was found as frequent as HPV 16 in cervical cancer (14) or even more frequently than HPV 16 (9, 15). We investigated the prevalent HPV types in healthy Indonesian women, described in a hospital-based case control study in **chapter 2** and described in a population-based study in **chapter 3**.

A total of 74 cervical carcinoma cases and 209 control women, recruited from the gynecological outpatient clinic of in the University Hospital Rumah sakit Cipto Mangunkusumo in Jakarta, were included. HPV was detected in 95.9% of the cases and in 25.4% of the controls and we calculated an OR for HPV infection of 69.5 for HPV prevalence in the cases. The high prevalence of HPV, found in our hospital-based control was in agreement with the high estimated incidence rate of cervical cancer in Indonesia (6, 16, 17). In our study population the detection rate of HPV 16 and HPV 18 in the case group were comparable, which we also found in the control group. These two findings suggest that the oncogenic potentials of HPV 16 and HPV

18 in Indonesia are similar. We supposed that the high incidence of HPV 18 in the control group compared to the world incidence of HPV 18, will be related to the high prevalence of HPV 18 in the general Indonesian population (**chapter 2**).

Based on the findings from this case-control study, we investigated the age-specific prevalence of HPV types among women in the Indonesian population and possible risk factors of HPV positivity, described in **chapter 3.**

Of 20.834 women 2686 random age stratified samples were taken and HPV was tested. In this population-based study, 91.2% of the women had never been screened before. The overall HPV prevalence was 11.4%, age-standardized to the world standard population 11.6%.
We found that in Indonesia HPV 16 and 18 are equally common in the general population, as they are in cervical cancer as described before. We also found that HPV 52 was the most prevalent type. In Indonesia different age-specific prevalence patterns were seen overall high in Jakarta and in Tasikmalaya and declining with age in Bali.

In the risk calculations, HPV positivity was found to be associated with a history of more than one sexual partner (OR 1.81 95% CI 1.31-2.51), as expected (this thesis), (1, 18-20). A small group of divorced women in Tasikmalaya and women with high daily income in Bali were associated with HPV positivity, which could also reflect sexual behavior **(chapter 3)**. Significant risk factors for cervical cancer in addition to HPV infection were young age at first intercourse, having a history of more than one sexual partner and high parity **(chapter 2).**

Screening and treatment

The need for secondary prevention programs remains as long as HPV vaccines are not yet available to all women in the world and do not yet provide a 100% coverage of all HPV types. Moreover, surveillance and management of women who haven't been vaccinated before the onset of sexual activity, is still needed.

Because of the high incidence rates in low- and middle-income countries, it is of major importance that the screening methods chosen are highly efficient, cost-effective and feasible in local circumstances. Screening using visual inspection with acetic acid (VIA) and treatment using cryotherapy have shown to meet these requirements and have shown to reduce incidence and mortality due to cervical cancer (21-28).

Based on these findings we set up the See and Treat project for cervical cancer screening described in **Chapter 4.**

To be effective in reducing incidence and mortality rates, screening programmes should focus on the target population. The target population for cervical (pre-) cancer are women aged 30-49 in whom the highest incidence in of (pre-)malignant lesions

are found (29-32). When financial resources are limited, focus on the target population should be intensified.

As our study describes a pilot project, the target population was 30-49 years old but was open to all women interested to participate. Our study population reinforces the importance of the target population as the highest prevalence of women with a positive VIA screening test was found in the high-risk group of women aged 30–49, accounting for 70.7% of positive cases.

The overall positive predictive value of VIA for histological diagnosis of ≥ CIN I and ≥ CIN II was 58.7% and 29.3%, respectively. The approximate specificity was 98.1%, and the detection rate for ≥ CIN I was 2.6% **(chapter 2)**. Our study gives insight in the use of VIA in the field conditions and its routine performance in health services in Indonesia. Our rates are comparable to population-based VIA screening programs in other high incidence areas as Tanzania (VIA positivity 3.8%), Bangladesh (4.8%) and Angola (6.6%) (33-35). The use of VIA and cryotherapy performed well in the described single-visit approach, and the acceptance of the procedure was high.

A major aspect in screening programmes is awareness: women have to be informed about cervical cancer, about the importance of screening, and about symptoms that could reveal cervical lesions. When these awareness programmes are adjusted to the culture of the population screened, the information will be more accepted by its public and will have more impact (36-38). To avoid unnecessary stigmatization, it is important to underline the fact that cervical cancer is a rare complication of a highly prevalent high-risk HPV type infection because of a failing immune system rather than a sexual transmitted disease as 85% of all women will be infected with HPV sometime during their lifetime (39).

Collaboration with existing health institutions is of major importance. In the See and Treat project, collaboration was set up with the Indonesian family welfare movement (Pembinaan Kesehahteraan Keluarga PKK), the Indonesian cancer foundation (Yayasan Kanker Indonesia YKI), and the University hospitals (Rumah sakit Cipto Manungkosumo in Jakarta Java and Rumah Sakit Sanglah in Den Pasar Bali).

This collaboration succeeded in reaching the target population, i.e., high-risk women who had limited access to health-care facilities and lived in low-resource settings **(chapter 4)**. When screening programmes are implemented in already existing health structures, the potential of sustainability increases. When the implementation succeeds on low scale it is easier to scale-up (40).

Screening with the optoelectronic device (Truscreen) is another alternative to conventional cytology screening. This device utilizes optical and electrical technology, it has the advantage of producing an immediate result, it is objective, requires low training efforts and does not need laboratory equipment. We assessed its performance in a perfectly regulated tertiary hospital setting and compared the results to liquid-based cytology and HPV testing. Truscreen demonstrated

comparable sensitivity to high quality cytology, and sensitivity and specificity approaching HPV DNA testing **(chapter 6)**. The device showed promising results in a hospital setting and should now be further tested in population based cervical cancer screening settings. A review based on Chinese studies showed a moderately good diagnostic accuracy (41), a Mexican study showed low sensitivity but high specificity (42). Truscreen can potentially become an important tool in the prevention of cervical cancer, particularly in low- and middle-income countries with resource-limited see and treat screening settings as results are directly available.

More screening tests and techniques are being used and developed. HPV DNA self-sampling is upcoming and has been reported to be of patient preference because of privacy and ease (43). Self-samples are accurate and it's an effective way to reach under screened women (44). Molecular tests, dual staining on cytological slides and more advanced visual inspection tests based on artificial intelligence or machine learning platforms are under research.

Cryotherapy

Excisional methods of treatment (LEEP/LLETZ) are very effective and standard of care in developed countries, and overall LEEP has a higher overall cure rate compared to cryotherapy (96.4% compared to 88.3% respectively, p=0.026) (45). The treatment is less suitable for See and Treat approaches in low- and middle-income countries as it is a multistep treatment procedure that requires electricity, anesthetics, and it is associated with higher complications rates due to post treatment bleeding and infection. It also carries an increased risk of preterm delivery (46-48).

The WHO guideline recommends to use cryotherapy in resource restrained settings (and LEEP when the lesions are not eligible for cryotherapy) (49),(50). Cryotherapy is a widely used treatment in a single visit approach setting during cervical cancer screening. It has proven to be safe, effective, easy to use, cheap and readily available in low resource areas (51-53). Treatment of premalignant lesions using thermal ablation has been evaluated as alternative to cryotherapy and shows comparable or better performance (54). Thermal ablation is a battery-powered, hand-held device, not requiring any gas which makes its usage in low resource settings easier. Immunomodulators and therapeutic vaccines might be future options to treat premalignant lesions but are now still in research setting.

The use of VIA and cryotherapy in the single-visit approach in low resource settings in Indonesia as described in **chapter 4**, performed well, and the acceptance of the therapy was high. There were no major side effects reported, although we might not have been fully informed as follow-up rates are limited. However, these findings are consistent with earlier performed studies on the safety, acceptability and feasibility of cryotherapy, where side effects were found to be rare (55, 56).

The overall positive predictive value of VIA for histological diagnosis of ≥ CIN I and ≥ CIN II was 58.7% and 29.3%, respectively, with consequent overtreatment rates of 41.3% and 70.7%, respectively (**chapter 4**). Although these overtreatment rates are high, we think these are acceptable as the morbidity associated with cryotherapy is very low and the overall benefit of treatment in reducing the risk of cervical cancer in high incidence areas is significant. In a large randomized trial in India, the intervention group (screening with VIA and treatment with cryotherapy if eligible) had a significant 25% reduction in cervical cancer mortality (HR 0.65 (0.47-0.89) compared to the control group after 7 years of follow up (57).

Only 47.4% of the initial population that received cryotherapy came for follow-up screening despite several attempts to retrieve them all. These low follow up rates underline the importance of the single visit approach in low- and middle-income countries (**chapter 4**).

Although many HPV infections are cleared spontaneously within 2 years (39, 58), we can conclude that cryotherapy does increase the HPV clearance rate **(chapter 5)**. Six months after cryotherapy we found an 80.3% clearance rate for type specific HPV infections. The most common persisting HPV types after cryotherapy were HPV 51, HPV 18, HPV16 and HPV 52.

HPV types 16, 31, 33, 35, 52, and 58 are phylogenetically related and grouped as clade a-9, while HPV types 18, 39, 45, 59, and 68 are phylogenetically related and grouped as clade a-7. The clades show differences in preferences with respect to the site of infection. The clade a-9 genotypes are more associated with squamous cell carcinomas (SCC), the clade a-7 genotypes are more commonly associated with adenocarcinomas (ADC) (59-63). Although most HPV types in these clades can lead to 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 (60, 62). As some HPV types are more associated with squamous cell tissue and others are more associated with glandular cell tissue, there might be a difference in persistence after cryotherapy.

We didn't find any difference in the risk of persistence between the HPV types that are associated with glandular cells (clade a-7) compared to those associated with squamous cells (clade a-9) **(chapter 5).** Consequently, it seems that the risk of undertreatment by cryotherapy of HPV clade a-7 infections because of the deeper location in the cervix, is relatively small. Probably part of the explanation lies with the cascade of immunological responses that cryotherapy induces.

The risk of HPV persistence after cryotherapy was significantly higher for low-risk HPV types compared to high-risk HPV types **(chapter 5)**. A possible explanation for the observed higher risk of persistence in low-risk HPV types is that after destroying the squamous columnar junction (SCJ) with cryotherapy, the high-risk HPV types will be cleared, and relatively more low-risk HPV types remain in the vagina and can be detected (64).

Cryotherapy is associated with a significant reduction in newly detected high-risk HPV infections. In a South African study, testing HPV after cryotherapy, women were 55% (95% CI 0.28–0.71) less likely to have a newly detected high-risk HPV infection compared to women in the no-treatment control group (65). Besides the immunological response induced by cryotherapy, infecting the SCJ after cryotherapy might be more difficult as the new SCJ migrates deeper into the endocervix.

Future, towards a world without cervical cancer

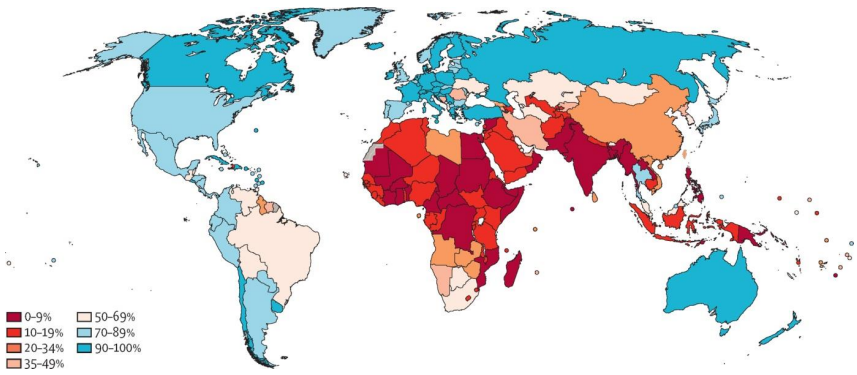
If prevention and screening programs are not implemented in low- and middle-income countries, more than 44 million women will be diagnosed with cervical cancer in the next 50 years (66).

In 2018 the WHO called for action to eliminate cervical cancer, by adopting the global strategy of cervical cancer as a public health problem to accelerate its elimination (67). It is the first time in history that a cancer has been named a public health problem. The points of actions are worldwide and at primary, secondary and tertiary level. The way to achieve this goal, eliminating cervical cancer, is referred to as 'the 90-70-90 targets' and should be accomplished by 2030. The first goal is that 90% of the girls should be fully vaccinated with an HPV vaccine by the age of 15. The second goal that 70% of the woman should be screened with a high-performance test at the age of 35 and again at the age of 45. And the third goal is that 90% of the identified premalignant lesions should be treated and 90% of the invasive cancer should be managed accordingly (67).

If these goals are achieved, it's possible to reduce in mortality from cervical cancer in low- and middle-income countries by the year 2030 by one third. If the strategy is implemented successfully worldwide, by the year 2130 more than 62 million women's lives will be saved which would reduce the mortality from cervical cancer by 99%. By then, cervical cancer will be a rare disease with an incidence of ≤ 4 per 100.000 women (66, 68).

Around 70% and 84% of cervical cancers can be prevented by Cervarix and Gardasil HPV vaccines (first generation), and about 90% of cervical cancers using Gardasil 9 (66). By 2017, globally 107 countries (37%) had introduced HPV vaccination in their national immunization program for girls, and 11 countries (6%) also for boys. However, by 2021, only 15% of the girls in the world are yet fully protected (69). To ensure long-term sustainability, HPV vaccine supply and access is of utmost importance. Gavi, the vaccine alliance plays an important role in assisting low- and middle- income countries setting up vaccination program (70).

In 65% of the countries worldwide screening program are in place, in 40% these are population-based, in 18% they reach the cover target of at least 70% (71). The estimated worldwide coverage of women screened at least once in their lifetime between the age of 30-49 years is 36%, which means 2 in 3 women aged 30-49 years have never been screened before, see the figure below for the worldwide distribution (72). In low resource settings premalignant lesions are mostly treated with cryotherapy, but thermal ablation is an upcoming modality. Facilities to treat more extensive premalignant lesions and cancers should be in place. Now, in 90% of the high-income countries, and in 15% of the middle- low- income countries have cancer surgery, radiation and chemotherapy facilities (WHO).



Ever in lifetime cervical cancer screening coverage in women aged 30-49 years in 2019 by country. Adapted from Bruni et al Lancet 2022 (72)

The recommendation of the WHO regarding see and treat strategies in low resource settings, used to be screening with VIA and treatment using cryotherapy, as we did in the screening project in Indonesia. Now is has been proven that the impact on incidence and mortality is even higher using HPV DNA testing followed by treatment, and the strategy recommended by the WHO has now been adjusted to HPV DNA screening (73-78).

If resources are sufficient, all positive HPV tests can be followed by a triage test (VIA, colposcopy, cytology, HPV16/18 analysis, p16/Ki67 dual staining) before treatment to reduce the amount of overtreatment (49). When resources are insufficient for a triage test, HPV testing alone followed by treatment may achieve greater health benefits (79, 80).

In resource-constrained settings, where screening with HPV DNA tests is not feasible, screening with VIA followed by treatment is still suggested (73). When the infrastructure for VIA screening has been set up, it can be easily changed into HPV screening when the resources would improve (81). The WHO has displayed 7

strategies for screening and treatment, as it remains of outmost importance that the strategy chosen is feasible in the local circumstances.

Overall, pilot projects to set up awareness, screening, treatment, call- and recall-systems, give insight in the possibilities and difficulties of local circumstances. Once the set up in the pilot project is optimized, implementation on national level is the ultimate goal in cervical cancer screening.

Organized population based screening program linked to feasible and affordable treatment for all stages of disease together with high HPV vaccination coverage, will be key towards a world without cervical cancer.

References

1. Cohen PA, Jhingran A, Oaknin A, Denny L. Cervical cancer. Lancet (London, England). 2019;393(10167):169-82. Epub 2019/01/15.
2. Vaccarella S, De Vries E, Sierra MS, Conway DI, Mackenbach JP. IARC Scientific Publications

Social inequalities in cancer within countries. In: Vaccarella S, Lortet-Tieulent J, Saracci R, Conway DI, Straif K, Wild CP, editors. Reducing social inequalities in cancer: evidence and priorities for research. Lyon (FR): International Agency for Research on Cancer

3. Denny L, de Sanjose S, Mutebi M, Anderson BO, Kim J, Jeronimo J, et al. Interventions to close the divide for women with breast and cervical cancer between low-income and middle-income countries and high-income countries. Lancet (London, England). 2017;389(10071):861-70. Epub 2016/11/07.
4. Vaccarella S, Lortet-Tieulent J, Plummer M, Franceschi S, Bray F. Worldwide trends in cervical cancer incidence: impact of screening against changes in disease risk factors. European journal of cancer (Oxford, England : 1990). 2013;49(15):3262-73. Epub 2013/06/12.
5. Aoki ES, Yin R, Li K, Bhatla N, Singhal S, Ocviyanti D, et al. National screening programs for cervical cancer in Asian countries. Journal of gynecologic oncology. 2020;31(3):e55. Epub 2020/04/09.
6. Aziz MF. Gynecological cancer in Indonesia. Journal of gynecologic oncology. 2009;20(1):8-10. Epub 2009/05/28.
7. Guan P, Howell-Jones R, Li N, Bruni L, de Sanjosé S, Franceschi S, et al. Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. Int J Cancer. 2012;131(10):2349-59. Epub 2012/02/11.
8. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. The New England journal of medicine. 2003;348(6):518-27. Epub 2003/02/07.
9. Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. Journal of the National Cancer Institute. 1995;87(11):796-802. Epub 1995/06/07.
10. de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. Int J Cancer. 2017;141(4):664-70. Epub 2017/04/04.
11. Clifford GM, Gallus S, Herrero R, Munoz N, Snijders PJ, Vaccarella S, et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet (London, England). 2005;366(9490):991-8. Epub 2005/09/20.
12. Franceschi S, Herrero R, Clifford GM, Snijders PJ, Arslan A, Anh PT, et al. Variations in the age-specific curves of human papillomavirus prevalence in women worldwide. Int J Cancer. 2006;119(11):2677-84. Epub 2006/09/23.
13. Bruni L, Diaz M, Castellsagué X, Ferrer E, Bosch FX, de Sanjosé S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. The Journal of infectious diseases. 2010;202(12):1789-99. Epub 2010/11/12.
14. Schellekens MC, Dijkman A, Aziz MF, Siregar B, Cornain S, Kolkman-Uljee S, et al. Prevalence of single and multiple HPV types in cervical carcinomas in Jakarta, Indonesia. Gynecologic oncology. 2004;93(1):49-53. Epub 2004/03/30.
15. Panigoro R, Susanto H, Novel SS, Hartini S, Sahiratmadja E. HPV genotyping linear assay test comparison in cervical cancer patients: implications for HPV prevalence and molecular epidemiology in a limited-resource area in Bandung, Indonesia. Asian Pacific journal of cancer prevention : APJCP. 2013;14(10):5843-7. Epub 2013/12/03.

16. Singh D, Vignat J, Lorenzoni V, Eslahi M, Ginsburg O, Lauby-Secretan B, et al. Global estimates of incidence and mortality of cervical cancer in 2020: a baseline analysis of the WHO Global Cervical Cancer Elimination Initiative. The Lancet Global health. 2023;11(2):e197-e206. Epub 2022/12/18.
17. Tjindarbumi D, Mangunkusumo R. Cancer in Indonesia, present and future. Japanese journal of clinical oncology. 2002;32 Suppl:S17-21. Epub 2002/04/18.
18. Okunade KS. Human papillomavirus and cervical cancer. Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology. 2020;40(5):602-8. Epub 2019/09/11.
19. Lenselink CH, Melchers WJ, Quint WG, Hoebers AM, Hendriks JC, Massuger LF, et al. Sexual behaviour and HPV infections in 18 to 29 year old women in the pre-vaccine era in the Netherlands. PloS one. 2008;3(11):e3743. Epub 2008/11/18.
20. Vaccarella S, Franceschi S, Herrero R, Munoz N, Snijders PJ, Clifford GM, et al. Sexual behavior, condom use, and human papillomavirus: pooled analysis of the IARC human papillomavirus prevalence surveys. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006;15(2):326-33. Epub 2006/02/24.
21. Visual inspection with acetic acid for cervical-cancer screening: test qualities in a primary-care setting. University of Zimbabwe/JHPIEGO Cervical Cancer Project. Lancet (London, England). 1999;353(9156):869-73. Epub 1999/03/27.
22. Denny L, Kuhn L, Pollack A, Wainwright H, Wright TC, Jr. Evaluation of alternative methods of cervical cancer screening for resource-poor settings. Cancer. 2000;89(4):826-33. Epub 2000/08/22.
23. Mandelblatt JS, Lawrence WF, Gaffikin L, Limpahayom KK, Lumbiganon P, Warakamin S, et al. Costs and benefits of different strategies to screen for cervical cancer in less-developed countries. Journal of the National Cancer Institute. 2002;94(19):1469-83. Epub 2002/10/03.
24. Gaffikin L, Blumenthal PD, Emerson M, Limpaphayom K. Safety, acceptability, and feasibility of a single-visit approach to cervical-cancer prevention in rural Thailand: a demonstration project. Lancet (London, England). 2003;361(9360):814-20. Epub 2003/03/19.
25. Gaffikin L, Lauterbach M, Blumenthal PD. Performance of visual inspection with acetic acid for cervical cancer screening: a qualitative summary of evidence to date. Obstetrical & gynecological survey. 2003;58(8):543-50. Epub 2003/07/30.
26. Sankaranarayanan R, Shastri SS, Basu P, Mahe C, Mandal R, Amin G, et al. The role of low-level magnification in visual inspection with acetic acid for the early detection of cervical neoplasia. Cancer detection and prevention. 2004;28(5):345-51. Epub 2004/11/16.
27. Goldie SJ, Gaffikin L, Goldhaber-Fiebert JD, Gordillo-Tobar A, Levin C, Mahe C, et al. Cost-effectiveness of cervical-cancer screening in five developing countries. The New England journal of medicine. 2005;353(20):2158-68. Epub 2005/11/18.
28. Sankaranarayanan R, Rajkumar R, Theresa R, Esmy PO, Mahe C, Bagyalakshmi KR, et al. Initial results from a randomized trial of cervical visual screening in rural south India. Int J Cancer. 2004;109(3):461-7. Epub 2004/02/13.
29. WHO Guidelines Approved by the Guidelines Review Committee. WHO guideline for screening and treatment of cervical pre-cancer lesions for cervical cancer prevention. Geneva: World Health Organization

30. Arbyn M, Castellsague X, de Sanjose S, Bruni L, Saraiya M, Bray F, et al. Worldwide burden of cervical cancer in 2008. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2011;22(12):2675-86. Epub 2011/04/08.
31. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet (London, England). 2007;370(9590):890-907. Epub 2007/09/11.
32. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127(12):2893-917.

33. Ngoma T, Muwonge R, Mwaiselage J, Kawegere J, Bukori P, Sankaranarayanan R. Evaluation of cervical visual inspection screening in Dar es Salaam, Tanzania. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2010;109(2):100-4. Epub 2010/02/16.
34. Nessa A, Hussain MA, Rahman JN, Rashid MH, Muwonge R, Sankaranarayanan R. Screening for cervical neoplasia in Bangladesh using visual inspection with acetic acid. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2010;111(2):115-8. Epub 2010/08/03.
35. Muwonge R, Manuel Mda G, Filipe AP, Dumas JB, Frank MR, Sankaranarayanan R. Visual screening for early detection of cervical neoplasia in Angola. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2010;111(1):68-72. Epub 2010/06/24.
36. Gelband H, Sankaranarayanan R, Gauvreau CL, Horton S, Anderson BO, Bray F, et al. Costs, affordability, and feasibility of an essential package of cancer control interventions in low-income and middle-income countries: key messages from Disease Control Priorities, 3rd edition. Lancet (London, England). 2016;387(10033):2133-44. Epub 2015/11/19.
37. http://www.who.int/cancer/media/en/cancer_cervical_37321.pdf RoaWc.
38. Sivaram S, Majumdar G, Perin D, Nessa A, Broeders M, Lynge E, et al. Population-based cancer screening programmes in low-income and middle-income countries: regional consultation of the International Cancer Screening Network in India. The Lancet Oncology. 2018;19(2):e113-e22. Epub 2018/02/08.
39. Schiffman M, Doorbar J, Wentzensen N, de Sanjose S, Fakhry C, Monk BJ, et al. Carcinogenic human papillomavirus infection. Nature reviews Disease primers. 2016;2:16086. Epub 2016/12/03.
40. Parham GP, Mwanahamuntu MH, Kapambwe S, Muwonge R, Bateman AC, Blevins M, et al. Population-level scale-up of cervical cancer prevention services in a low-resource setting: development, implementation, and evaluation of the cervical cancer prevention program in Zambia. PloS one. 2015;10(4):e0122169. Epub 2015/04/18.
41. Yang H, Zhang X, Hao Z. The diagnostic accuracy of a real-time optoelectronic device in cervical cancer screening: A PRISMA-compliant systematic review and meta-analysis. Medicine. 2018;97(29):e11439. Epub 2018/07/20.
42. Salazar-Campos JE, González-Enciso A, Díaz-Molina R, Lara-Hernández ME, Coronel-Martínez J, Pérez-Plasencia C, et al. Cervicouterine Cancer Screening - TruScreen™ vs. Conventional Cytology: Pilot Study. Journal of cytology. 2018;35(3):143-8. Epub 2018/08/10.
43. Nelson EJ, Maynard BR, Loux T, Fatla J, Gordon R, Arnold LD. The acceptability of self-sampled screening for HPV DNA: a systematic review and meta-analysis. Sexually transmitted infections. 2017;93(1):56-61. Epub 2017/01/20.
44. Arbyn M, Smith SB, Temin S, Sultana F, Castle P. Detecting cervical precancer and reaching underscreened women by using HPV testing on self samples: updated meta-analyses. BMJ (Clinical research ed). 2018;363:k4823. Epub 2018/12/07.
45. Chirenje ZM, Rusakaniko S, Akino V, Mlingo M. A randomised clinical trial of loop electrosurgical excision procedure (LEEP) versus cryotherapy in the treatment of cervical intraepithelial neoplasia. Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology. 2001;21(6):617-21. Epub 2003/01/11.
46. Bruinsma FJ, Quinn MA. The risk of preterm birth following treatment for precancerous changes in the cervix: a systematic review and meta-analysis. BJOG : an international journal of obstetrics and gynaecology. 2011;118(9):1031-41. Epub 2011/04/01.
47. Arbyn M, Kyrgiou M, Simoens C, Raifu AO, Koliopoulos G, Martin-Hirsch P, et al. Perinatal mortality and other severe adverse pregnancy outcomes associated with treatment of cervical intraepithelial neoplasia: meta-analysis. BMJ (Clinical research ed). 2008;337:a1284. Epub 2008/09/20.
48. Kyrgiou M, Koliopoulos G, Martin-Hirsch P, Arbyn M, Prendiville W, Paraskevaidis E. Obstetric outcomes after conservative treatment for intraepithelial or early invasive cervical lesions:

systematic review and meta-analysis. Lancet (London, England). 2006;367(9509):489-98. Epub 2006/02/14.
49. Santesso N, Mustafa RA, Schunemann HJ, Arbyn M, Blumenthal PD, Cain J, et al. World Health Organization Guidelines for treatment of cervical intraepithelial neoplasia 2-3 and screen-and-treat strategies to prevent cervical cancer. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2016;132(3):252-8. Epub 2016/02/13.
50. prevention WGfsatoplfcc, http://www.who.int/reproductivehealth/publications/cancers/screening_and_treatment_of_precancerous_lesions/en/.
51. Basu P, Taghavi K, Hu SY, Mogri S, Joshi S. Management of cervical premalignant lesions. Current problems in cancer. 2018;42(2):129-36. Epub 2018/02/13.
52. Martin-Hirsch PP, Paraskevaidis E, Bryant A, Dickinson HO. Surgery for cervical intraepithelial neoplasia. The Cochrane database of systematic reviews. 2013(12):Cd001318. Epub 2013/12/05.
53. Alliance for Cervical Cancer PreventionACCP. Effectiveness s, and acceptability of cryotherapy. A systematic literature review. Cervical cancer prevention issues in depth, no 10 (2003).
54. de Fouw M, Oosting RM, Rutgrink A, Dekkers OM, Peters AAW, Beltman JJ. A systematic review and meta-analysis of thermal coagulation compared with cryotherapy to treat precancerous cervical lesions in low- and middle-income countries. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2019;147(1):4-18. Epub 2019/07/06.
55. Castle PE, Murokora D, Perez C, Alvarez M, Quek SC, Campbell C. Treatment of cervical intraepithelial lesions. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2017;138 Suppl 1:20-5. Epub 2017/07/12.
56. WHO Guidelines Approved by the Guidelines Review Committee. WHO Guidelines: Use of Cryotherapy for Cervical Intraepithelial Neoplasia. Geneva: World Health Organization

57. Sankaranarayanan R, Esmy PO, Rajkumar R, Muwonge R, Swaminathan R, Shanthakumari S, et al. Effect of visual screening on cervical cancer incidence and mortality in Tamil Nadu, India: a cluster-randomised trial. Lancet (London, England). 2007;370(9585):398-406. Epub 2007/08/07.
58. Schiffman M, Wentzensen N. Human papillomavirus infection and the multistage carcinogenesis of cervical cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2013;22(4):553-60. Epub 2013/04/04.
59. Seoud M, Tjalma WA, Ronsse V. Cervical adenocarcinoma: moving towards better prevention. Vaccine. 2011;29(49):9148-58. Epub 2011/10/11.
60. Bulk S, Berkhof J, Bulkmans NW, Zielinski GD, Rozendaal L, van Kemenade FJ, et al. Preferential risk of HPV16 for squamous cell carcinoma and of HPV18 for adenocarcinoma of the cervix compared to women with normal cytology in The Netherlands. British journal of cancer. 2006;94(1):171-5. Epub 2006/01/13.
61. Clifford G, Franceschi S. Members of the human papillomavirus type 18 family (alpha-7 species) share a common association with adenocarcinoma of the cervix. Int J Cancer. 2008;122(7):1684-5. Epub 2007/12/07.
62. Li N, Franceschi S, Howell-Jones R, Snijders PJ, Clifford GM. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. Int J Cancer. 2011;128(4):927-35. Epub 2010/05/18.
63. de Villiers EM. Cross-roads in the classification of papillomaviruses. Virology. 2013;445(1-2):2-10. Epub 2013/05/21.
64. Castle PE, Rodriguez AC, Porras C, Herrero R, Schiffman M, Gonzalez P, et al. A comparison of cervical and vaginal human papillomavirus. Sexually transmitted diseases. 2007;34(11):849-55. Epub 2007/07/11.

65. Taylor S, Wang C, Wright TC, Denny L, Tsai WY, Kuhn L. Reduced acquisition and reactivation of human papillomavirus infections among older women treated with cryotherapy: results from a randomized trial in South Africa. BMC medicine. 2010;8:40. Epub 2010/07/01.
66. Simms KT, Steinberg J, Caruana M, Smith MA, Lew JB, Soerjomataram I, et al. Impact of scaled up human papillomavirus vaccination and cervical screening and the potential for global elimination of cervical cancer in 181 countries, 2020-99: a modelling study. The Lancet Oncology. 2019;20(3):394-407. Epub 2019/02/24.
67. Organization WH. Accelerating cervical cancer elimination. Executive board 144th session provisional agenda item 65 EB144/28. 2018.
68. Canfell K, Kim JJ, Brisson M, Keane A, Simms KT, Caruana M, et al. Mortality impact of achieving WHO cervical cancer elimination targets: a comparative modelling analysis in 78 low-income and lower-middle-income countries. Lancet (London, England). 2020;395(10224):591-603. Epub 2020/02/03.
69. Bruni L, Saura-Lázaro A, Montoliu A, Brotons M, Alemany L, Diallo MS, et al. HPV vaccination introduction worldwide and WHO and UNICEF estimates of national HPV immunization coverage 2010-2019. Preventive medicine. 2021;144:106399. Epub 2021/01/04.
70. GAVI. https://www.gavi.org/.
71. Torode J, Kithaka B, Chowdhury R, Simelela N, Cruz JL, Tsu VD. National action towards a world free of cervical cancer for all women. Preventive medicine. 2021;144:106313. Epub 2021/03/09.
72. Bruni L, Serrano B, Roura E, Alemany L, Cowan M, Herrero R, et al. Cervical cancer screening programmes and age-specific coverage estimates for 202 countries and territories worldwide: a review and synthetic analysis. The Lancet Global health. 2022;10(8):e1115-e27. Epub 2022/07/16.
73. WHO. WHO guidelines for screening and treatment of cervical pre-cancer lesions for cervical cancer prevention, second edition Web Annex B: Evidence to Decision Tables. 2021.
74. Denny L, Kuhn L, Hu CC, Tsai WY, Wright TC, Jr. Human papillomavirus-based cervical cancer prevention: long-term results of a randomized screening trial. Journal of the National Cancer Institute. 2010;102(20):1557-67. Epub 2010/10/05.
75. Sankaranarayanan R, Nene BM, Shastri SS, Jayant K, Muwonge R, Budukh AM, et al. HPV screening for cervical cancer in rural India. The New England journal of medicine. 2009;360(14):1385-94. Epub 2009/04/03.
76. Ronco G, Dillner J, Elfstrom KM, Tunesi S, Snijders PJ, Arbyn M, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet (London, England). 2014;383(9916):524-32. Epub 2013/11/07.
77. Arbyn M, Ronco G, Anttila A, Meijer CJ, Poljak M, Ogilvie G, et al. Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. Vaccine. 2012;30 Suppl 5:F88-99. Epub 2012/12/05.
78. Bouvard V, Wentzensen N, Mackie A, Berkhof J, Brotherton J, Giorgi-Rossi P, et al. The IARC Perspective on Cervical Cancer Screening. The New England journal of medicine. 2021;385(20):1908-18. Epub 2021/11/11.
79. Campos NG, Jeronimo J, Tsu V, Castle PE, Mvundura M, Kim JJ. The Cost-Effectiveness of Visual Triage of Human Papillomavirus-Positive Women in Three Low- and Middle-Income Countries. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2017;26(10):1500-10. Epub 2017/07/16.
80. Nguyen DTN, Simms KT, Keane A, Mola G, Bolnga JW, Kuk J, et al. Towards the elimination of cervical cancer in low-income and lower-middle-income countries: modelled evaluation of the effectiveness and cost-effectiveness of point-of-care HPV self-collected screening and treatment in Papua New Guinea. BMJ global health. 2022;7(3). Epub 2022/03/05.
81. Sankaranarayanan R, Qiao YL, Keita N. The next steps in cervical screening. Women's health (London, England). 2015;11(2):201-12. Epub 2015/03/18.

Chapter 8

Dutch summary, Nederlandse samenvatting

Dutch summary, Nederlandse samenvatting

Baarmoederhalskanker

Met 604.000 nieuwe gevallen van kanker per jaar, is baarmoederhalskanker is de derde meest voorkomende kanker en de vierde meest voorkomende oorzaak van sterfte onder vrouwen wereldwijd. De meeste gevallen van baarmoederhalskanker worden gediagnosticeerd in een verder gevorderd stadium van ziekte wat de overlevingskansen verslechterd. In 2020 zijn wereldwijd 342.000 vrouwen aan baarmoederhalskanker overleden.

Het voorkomen van baarmoederhalskanker neemt toe vanaf een leeftijd van 35-40 jaar en bereikt het maximum op een leeftijd van 50-60 jaar. Dat betekent dat baarmoederhalskanker vrouwen meestal treft in hun fertiele levensfase wanneer ze een centrale rol hun gezin met vaak jonge kinderen hebben. Naast de impact die baarmoederhalskanker heeft op de individuele gezinnen, heeft het ook impact op de maatschappij, daar bijvoorbeeld wereldwijd 75% van de voedselproductie tot stand komt door het werk van vrouwen.

De incidentie van baarmoederhalskanker is in lage- en middeninkomenslanden het hoogst, ongeveer 90% van alle nieuwe baarmoederhalskanker gevallen ontstaat daar. Baarmoederhalskanker is hier na borstkanker de meest voorkomende kanker bij vrouwen. De hoogste incidentie is in West-, Oost- en Zuid-Afrika, Zuid-Centraal Azië en Zuid-Amerika. De reden dat de incidentie daar zo hoog is, is omdat het moeilijk is gebleken om goedlopende landelijke screenings programma's op te zetten. Vaak is de infrastructuur van de gezondheidszorg niet toereikend en zijn er niet genoeg financiële middelen, human resources en medische faciliteiten.

Ontstaan van baarmoederhalskanker

Premaligne afwijkingen aan de baarmoederhals en baarmoederhalskanker worden veroorzaakt door het persisteren van een hoog risico type humaan papillomavirus (HPV). Het HPV is onder mannen en vrouwen een veelvoorkomend sexueel overdraagbaar virus, het risico voor een vrouw om een keer in haar leven geïnfecteerd te raken met het virus is 85%. De infectie komt het meest voor in de eerste 10 jaar van sexuele activiteit waarbij de hoogste prevalentie wordt gezien onder de 25 jaar. De meeste infecties met het HPV-virus zijn van voorbijgaande aard, zijn asymptomatisch, veroorzaken geen afwijkingen en worden door het lichaam in 1-2 jaar geklaard.

De HPV types worden onderverdeeld in laag-risico, niet oncogene typen die goedaardige genitale afwijkingen veroorzaken (condylomata accuminata) en hoog-

risico, oncogene typen die premaligne en maligne afwijkingen kunnen veroorzaken. Wanneer een infectie met hoog-risico HPV persisteert kan deze in 5-10 jaar premaligne afwijkingen veroorzaken door veranderingen aan het epitheel van de baarmoedermond. Over het algemeen duurt het ontstaan van baarmoederhalskanker 12-15 jaar vanaf het moment van infectie maar er zijn ook veronderstellingen dat klinisch relevante afwijkingen 2-3 jaar na een infectie kunnen ontstaan.

Het aantal sexuele partners en de leeftijd van sexueel actief worden, worden geassocieerd met een verhoogd risico op het in aanraking komen met een HPV-infectie. Roken veroorzaakt door de stof cotinine een verminderde afweer op cervicaal niveau, waardoor een HPV-infectie makkelijker persisteert.
Andere factoren die het risico op persisteren van de HPV-infectie verhogen zijn het langer dan 5 jaar gebruiken van orale anticonceptiva, hoge pariteit (veel kinderen hebben gekregen) en het eerder hebben doorgemaakt van een sexueel overdraagbare aandoening (SOA). Vrouwen die geïnfecteerd zijn met het humaan immunodeficiëntie virus (HIV) en vrouwen die immunosuppressiva gebruiken hebben een hogere kans op het persisteren van HPV-infectie en het verder ontwikkelen van premaligne en maligne afwijkingen.

Er zijn meer dan 100 verschillende soorten HPV typen geïdentificeerd waarvan er ongeveer 40 het genitaal kunnen infecteren. Het voorkomen van het HPV, de soorten HPV en de distributie van die soorten verschilt substantieel tussen populaties. De meeste baarmoederhalskanker worden veroorzaakt door 15 typen hoog risico HPV, waarvan wereldwijd HPV 16 en HPV 18 het meest voorkomend zijn en respectievelijk 54.6% en 15.8% van alle baarmoederhalskanker veroorzaken.

Dit proefschrift
In dit proefschrift zijn een aantal aspecten van HPV en screening naar baarmoederhalskanker in Indonesië onderzocht, een laag inkomensland met een hoge incidentie van baarmoederhalskanker. Allereerst zijn er twee onderzoeken gedaan naar de meest voorkomende humaan papillomavirus typen in Indonesië, een in een ziekenhuis setting en een in een bevolkingsonderzoek setting **(hoofdstuk 2 en 3)**. Daarna wordt een manier van baarmoederhalskanker screening beschreven waarbij gebruik wordt gemaakt van een single visit approach: screening met azijnzuur (visual inspection with acetic acid) en indien nodig, in dezelfde sessie behandeling met cryotherapie **(hoofdstuk 4).** Vervolgens hebben we gekeken welke HPV typen persisteren na behandeling met cryotherapie **(hoofdstuk 5).** In het laatste hoofdstuk wordt een onderzoek beschreven dat baarmoederhalskanker screening met een opto-electronisch instrument vergelijkt met HPV testen en cytologie **(hoofdstuk 6)**.

In de discussie worden de resultaten van de verschillende onderzoeken en overwegingen voor de toekomst besproken.

HPV voorkomen

Uit onderzoek naar HPV typen in baarmoederhalskanker samples uit Indonesië was gebleken dat het percentage HPV 18 hoger lag dan wereldwijd werd beschreven. Om te zien of dit in de populatie ook het geval was werd in **hoofdstuk 2** een case-control study uitgevoerd op 74 patiënten met baarmoederhalskanker in het universiteitsziekenhuis in Jakarta, Java, Indonesië en 209 gezonde controles die werden gerecruteerd van de polikliniek gynaecologie van hetzelfde ziekenhuis. Op de samples van alle vrouwen werd een HPV typering gedaan met line probe assay (LiPA) en er werden vragenlijsten afgenomen om potentiële risicofactoren voor baarmoederhalskanker te achterhalen. HPV werd in 95.9% van de baarmoederhalskanker groep en in 25.4% van de controle groep gedetecteerd. In de controle groep was 13.4% geïnfecteerd met een hoog risico HPV type. HPV 16 werd bij 35% van de baarmoederhalskankergevallen en bij 1.9% van de controle groep gedetecteerd, voor HPV 18 was dat in 28% van de kankers en in 2.4% van de controle groep. Deze uitkomsten suggereren dat de potentie om kanker te veroorzaken voor HPV 16 en HPV 18 in Indonesië gelijk zijn. Op jonge leeftijd sexueel actief worden, verschillende sexuele partners en het hebben van veel kinderen bleken significante risicofactoren voor het krijgen van baarmoederhalskanker. We concludeerden dat de hoge prevalentie van HPV 18 in baarmoederhalskanker in Indonesië wordt veroorzaakt door de hoge prevalentie van HPV 18 in de Indonesische populatie.

In **hoofdstuk 3** wordt onderzoek beschreven naar het leeftijdsspecifiek voorkomen van HPV in de Indonesische populatie en naar risicofactoren op het hebben van een HPV infectie. Er werden 2686 vrouwen uit Jakarta, Tasikmalaya en Bali geincludeerd in de leeftijd van 15-70 jaar. De prevalentie van HPV was 11.4%, leeftijds gestandaardiseerd naar de standaard wereld populatie was dat 11.6%. De meest voorkomende typen die werden gedetecteerd waren HPV 52, HPV 16, HPV 18 en HPV 39, respectievelijk 23.2, 18.0, 16.1 en 11.8% van de hoog risico typen. In 20.7% van de infecties werden verschillende typen geïdentificeerd. Er werden verschillende patronen herkend in het leeftijdsspecifiek voorkomen van HPV: In Jakarta en in Tasikmalaya was het in alle leeftijdscategorieën hoog, in Bali nam de prevalentie met het stijgen van de leeftijd af. Het aantal malen dat vrouwen waren getrouwd werd het meest geassocieerd met het detecteren van een HPV infectie (OR 1.81 95% CI 1.31-2.51). Opmerkelijk is dat in Indonesië in baarmoederhalskanker maar ook in de populatie HPV 18 evenveel voorkomt als HPV 16. Verder bleek dat HPV 52 het meest voorkomende type is de populatie, dit is een belangrijk gegeven om mee te

nemen wanneer een profylactisch HPV vaccin geïntroduceerd zou worden in Indonesië.

Screening en behandeling

Met het screening op voorlopers van baarmoederhalskanker, premaligne afwijkingen, en de behandeling daarvan wordt het aantal gevallen van kanker gereduceerd. De effectiviteit van screening wordt vertaald in het afnemen van het aantal nieuwe gevallen van baarmoederhalskanker en het afnemen van de mortaliteit. Het grootste effect is gesorteerd door het opzetten van georganiseerde screeningsprogramma's; het systematisch oproepen van vrouwen voor screening en zo nodig behandeling met follow up.

De grootste afname van de incidentie van baarmoederhalskanker wordt bereikt als tenminste 80% van een populatie wordt bereikt.

De screening zelf is het meest effectief als wordt gescreend op het moment dat de prevalentie van de ziekte het hoogst is, in het geval van premaligne baarmoederhals afwijkingen is dat tussen de 30-49 jaar, gemiddeld ongeveer 10 jaar voordat er echt kanker ontstaat.

In Nederland worden alle vrouwen tussen de 30 en 60 jaar ieder 5 jaar opgeroepen voor een uitstrijk in het bevolkingsonderzoek, er wordt gescreend door middel van cytologie met daarbij HPV testen. Indien er afwijkingen zijn wordt er een colposcopie verricht waarbij biopten worden genomen en indien nodig volgt behandeling door middel van een lisexcisie (LLETZ). Follow up wordt cytologisch 6, 12 en 24 maanden na de ingreep verricht en indien alle drie de uitslagen goed zijn gaat verdere vervolging middels het bevolkinsonderzoek.

In lage- en middeninkomenslanden is het moeilijk gebleken georganiseerde cytologische screening op te zetten aangezien er vaak gebrek is aan financiële middelen, goede infrastructuur in de zorg, medische professionals en labarotoriumvoorzieningen met een goed kwaliteitscontrole systeem. Omdat de incidentie van baarmoederhalskanker juist hier hoog is, hebben we onderzoek gedaan naar een simpele, doeltreffende, kosteneffectieve en veilige methode van screening en behandeling van premaligne afwijkingen.

In **hoofdstuk 4** wordt het See and Treat single visit baarmoederhalskanker screeningsproject beschreven dat werd opgezet in low resource gebieden in Jakarta, Tasikmalaya op Java en op Bali, Indonesië. Het is een cross-sectionele studie waarbij de bruikbaarheid van het screenen met azijnzuur (visual inspection with acetic acid VIA), het nemen van histologie en het behandelen met cryotherapie werd geevalueerd. Daartoe werd een samenwerking aangegaan met de universiteitsziekenhuizen in de betreffende regio's en met bestaande

gezondheidszorginstellingen in Indonesië. Vrouwen in geselecteerde regio's met een beperkte toegang tot gezondheidszorg werden geïnformeerd over baarmoederhalskanker en de gevolgen daarvan. Zij werden uitgenodigd om mee te doen aan het baarmoederhals screeningsprogramma dat voor de gelegenheid werd opgezet in hun dorp. Ze werden gescreend op afwijkingen aan de baarmoedermond door middel van VIA. Indien de test positief was, werd een biopt genomen van de afwijking en werden zij in dezelfde sessie behandeld met cryotherapie (indien de afwijking daarvoor geschikt was). Na 6 maanden werd follow-up verricht met VIA en cytologie. Als vrouwen werden gediagnosticeerd met baarmoederhalskanker of als daar verdenking op was, werden ze doorverwezen naar de universiteitsziekenhuizen waarmee werd samengewerkt. In totaal werden de onderzoeksresultaten van 22.040 vrouwen geëvalueerd van wie 92.7% nog nooit eerder gescreend was. De screening met azijnzuur was in 4.4% van de gevallen positief, de positief voorspellende waarde van de VIA om een premaligne afwijking aan de baarmoedermond te detecteren was 58.7%, de bij benadering berekende specificiteit was 98.1%. Het opzetten van een screeningsprogramma met behulp van reeds bestaande gezondheidsinstellingen in Indonesië bleek zeer effectief in het bereiken van vrouwen die normaal gesproken blijven verstoken van gezondheidszorg. De manier van screenen en de behandeling bleek zeer bruikbaar en effectief in deze setting. We concludeerden dat deze manier van screening en behandeling een veelbelovende manier is om het aantal gevallen van baarmoederhalskanker in Indonesië terug te dringen.

Binnen de HPV typen zijn verschillende groepen te onderscheiden die op basis van biologische eigenschappen op elkaar lijken, dit worden clades genoemd.
Clade a9 (HPV type 16, 31, 33, 35, 52, 58) heeft meer affiniteit met plaveiselepitheel en wordt meer geassocieerd met plaveiselcelcarcinomen, clade a 7 (HPV type 18, 36, 45, 59, 68) heeft meer affiniteit met cilindrisch epitheel en wordt meer geassocieerd met adenocarcinomen.
Er zijn zorgen dat cryotherapie minder effectief zou zijn voor afwijkingen die uitgaan van het cilinderepitheel omdat deze dieper gelegen kunnen zijn. In **hoofdstuk 5** wordt het persisteren van HPV typen onderzocht na een behandeling met cryotherapie, met speciale interesse voor HPV typen die geassocieerd worden met cilinderepitheel afwijkingen.
Van 367 vrouwen die een behandeling met cryotherapie ondergingen was een sample voor HPV-bepaling beschikbaar van voor en van 6 maanden na de behandeling. De samples werden getest op de aanwezigheid en type HPV met PCR en INNO-line probe assay (LiPA). Het percentage waarbij 6 maanden na de cryotherapie geen HPV meer aanwezig was, was 80.3%. De kans dat een laag-risico HPV type persisteerde was significant groter vergeleken met een hoog-risico HPV type (relatief risico 3.3 95% CI interval 1.66-6.67). De HPV typen die het meest

frequent persisteerden na behandeling waren HPV 18, 51, 52. Er werd geen verschil gezien in het persisteren van hoog-risico HPV typen die meer geassocieerd zijn met cilindrisch epitheel in vergelijking met HPV typen die meer geassocieerd zijn met plaveiselepitheel (relatief risico 0.63, 95% CI 0.12-3.29). Concluderend lijkt het effectief HPV te eradiceren door middel van cryotherapie, inclusief de typen die meer geassocieerd zijn met cilinderepitheel cellen. Vrouwen die na cryotherapie een persisterende hoog-risico HPV infectie hebben, moeten onder controle blijven omdat zij een verhoogde kans hebben het opnieuw ontstaan van (pre-) maligne afwijkingen.

In **hoofdstuk 6** hebben we gekeken naar screening met een opto-elektronisch apparaat, Truscreen, een ander alternatief voor cytologische screening. Truscreen heeft het voordeel dat het direct een uitslag geeft, dat het objectief is, dat het een kleine leercurve heeft en dat het geen verdere laboratoriumbepalingen behoeft. Dit apparaat gebruikt optische en elektrische technologie om uiteindelijk een uitslag te geven: afwijkend of niet afwijkend.
In een universitaire ziekenhuis setting werd gekeken naar de bruikbaarheid van het Truscreen apparaat en hebben we de resultaten vergeleken met de resultaten van HPV testen en liquid based cytologie. De resultaten van Truscreen lieten een vergelijkbare sensitiviteit zien als hoogkwalitatieve cytologie, en een sensitiviteit en specificiteit vergelijkbaar met HPV testen. De resultaten in deze ziekenhuis setting zijn veelbelovend, de volgende stap is het testen van de bruikbaarheid in bevolkingsonderzoeken in lage- en middeninkomenslanden. Als deze resultaten in die setting ook kunnen worden behaald, zou het een belangrijke bijdrage kunnen leveren aan screening met een See- and Treat approach omdat de resultaten objectief en direct beschikbaar zijn.

Overwegingen voor de toekomst
Als er geen maatregelen worden genomen om landelijke baarmoederhalsscreeningsprojecten op te zetten in lage- en middeninkomenslanden, zullen de komende 50 jaar meer dan 44 miljoen vrouwen gediagnosticeerd worden met baarmoederhalskanker.

Om het elimineren van baarmoederhalskanker te bewerkstelligen en te versnellen, heeft in 2018 de World Health Organization (WHO) baarmoederhalskanker als een volksgezondheidsprobleem bestempeld. Het is de eerste keer in de geschiedenis dat een kankersoort als volksgezondheidsprobleem wordt bestempeld.
De actiepunten die zijn gesteld, gelden voor de hele wereld en acteren op 3 niveaus: 1. vaccinatie, 2. screening en 3. behandeling.
De strategie van de WHO om baarmoederhalskanker te elimineren zou in 2030 in ieder land moeten zijn geïmplementeerd en worden de 90-70-90 doelen genoemd.

Het eerste doel is dat 90% van alle meisjes volledig gevaccineerd moet zijn met een HPV-vaccin voordat ze 15 jaar oud zijn. Het tweede doel is dat 70% van alle vrouwen op 35 en 45-jarige leeftijd met een goede test gescreend moet zijn. Het derde doel is dat 90% van de geïdentificeerde premaligne afwijkingen behandeld moet zijn en 90% van de geidentificeerde baarmoederhalskanker gevallen het juiste behandeltraject krijgt.

Als deze doelen voor 2030 worden behaald, is het in lage- en middeninkomenslanden mogelijk om een derde van alle baarmoederkanker gevallen te voorkomen. Wanneer de strategie wereldwijd met succes geïmplementeerd zou worden, zijn er over 100 jaar, in het jaar 2130, meer dan 62 miljoen vrouwen gered van baarmoederhalskanker, wat de incidentie met 99% zou reduceren. Dat zou baarmoederhalskanker een zeldzame ziekte maken met een incidentie van ≤ 4 per 100.000 vrouwen.

Ongeveer 70-84% van de baarmoederhalskanker gevallen kunnen worden voorkomen door het gebruik van de eerste generatie HPV-vaccines Cervarix en Gardasil, dit is ongeveer 90% wanneer het nieuwere vaccin Gardasil 9 wordt gebruikt. In 2017, hadden wereldwijd 107 landen (37%) HPV-vaccinaties voor meisjes opgenomen in hun landelijk vaccinatie programma en 11 landen (6) ook voor jongens. Maar in 2021, was maar 15% van alle meisjes in de wereld volledig gevaccineerd. Om de duurzaamheid van deze vaccinatieprogramma's te waarborgen is de toegang tot vaccinaties en de aanvoer van vaccinaties van grootste belang. De "Global Alliance for Vaccines and Immunizations" (GAVI), speelt een belangrijke rol in het opzetten van vaccinatieprogramma’s in lage- en middeninkomenslanden.

Wereldwijd, heeft 65% van de landen een baarmoederhalskanker screenings programma, 40% daarvan is bevolkingsonderzoek en in 18% wordt de beoogde 70% dekkingsgraad behaald. Het geschatte percentage van vrouwen dat wereldwijd ooit in haar leven is gescreend in de leeftijd van 30-49 jaar, is 36%. Dit betekent dat 2/3 van de vrouwen wereldwijd nog nooit gescreend is.
In lage- en middeninkomenslanden worden premaligne afwijkingen meestal met cryotherapie behandeld, al is thermal ablation een methode die aan terrein wint. Bij thermal ablation worden de afwijkende cellen behandeld door middel van verhitting met behulp van elektriciteit.
Naast een goede behandeling voor premaligne afwijkingen, moeten er ook goede mogelijkheden zijn om uitgebreidere premaligne afwijkingen en baarmoederhalskanker te behandelen. In de huidige situatie in de wereld, heeft 90% van de hoog inkomenslanden en 15% van de lage- en middeninkomenslanden

adequate mogelijkheden voor behandeling met chirurgie, radiotherapie en chemotherapie.

De aanbevelingen van de WHO wat betreft See en Treat strategieën in lage- en middeninkomenslanden was voorheen altijd screening met azijnzuur (visual inspection with acetic acid VIA) en behandeling met cryotherapie zoals we in Indonesië hebben gedaan. Ondertussen is aangetoond dat de impact op incidentie en mortaliteit nog hoger is als er gebruik wordt gemaakt van screening met HPV DNA testen waarna behandeling volgt. De aanbeveling van de WHO is daarop aangepast naar: "waar mogelijk screening met HPV DNA testen".

De WHO heeft 7 strategieën voor screening en behandeling uitgewerkt, zodat voor iedere lokale omstandigheid een passende strategie bestaat.
Als de middelen toereikend zijn, kan na een positieve HPV DNA screenings test een triage test worden ingezet. Zo kunnen de vrouwen die echt behandeling nodig hebben verder worden geïdentificeerd en zo kan het aantal overbehandelingen worden gereduceerd. Bij het ontbreken van een triage test, is het behandelen van alle vrouwen met een positieve HPV DNA test geïndiceerd. Wanneer de middelen niet toereikend zijn om met HPV DNA testen te screenen, dan luidt het advies van de WHO nog steeds om met VIA te screenen en de positieve gevallen te behandelen. Als de logistiek voor VIA screening met behandeling is geïmplementeerd, is dit eenvoudig om te zetten naar HPV DNA testen wanneer daar de mogelijkheden later wel voor zouden zijn.

Samenvattend geven pilotprojecten inzicht in de mogelijkheden en moeilijkheden bij het opzetten van screeningsprogramma's waarbij awareness, screening, behandeling, verwijzing en follow up systemen in kaart worden gebracht. Wanneer een pilotproject goed functioneert, is uitbereiding en implementatie naar nationaal niveau het uiteindelijke doel.

Betaalbare, goed georganiseerde bevolkingsonderzoeken met daaraan gekoppeld goede behandeling voor alle stadia van ziekte, zijn in combinatie met een hoge dekkingsgraad van HPV-vaccinatie uiteindelijk de sleutel voor een wereld zonder baarmoederhalskanker.

Abbreviations

ADC	Adenocarcinoma
AIS	Adenocarcinoma in situ
ASC-US significance	Atypical squamous cells of undetermined
CIN	Cervical intraepithelial neoplasia
CO_2	Carbon dioxide
HC2	Hybrid Capture 2
HIV	Human immunodeficiency virus
hr HPV	High risk human papillomavirus
HSIL	High-grade squamous intra-epithelial lesion
LCR	Long control region
LEEP	Loop electrosurgical excision procedure
LLETZ	Large loop excision of the transformation zone
LSIL	Low-grade squamous intraepithelial lesions
N_2O	Nitrous oxide
ORF	Open reading frame
SCC	Squamous cell carcinoma
SCJ	Squamous columnar junction
TZ	Transformation zone
VIA	Visual inspection with acetic acid
VIAM magnification	Visual inspection with acetic acid using
VILI	Visual inspection with lugol's iodine (VILI)
VLP	Virus-like-particles
YKI foundation	Yayasan Kanker Indonesia Indonesian cancer

About the author

Jessica Vet was born on the 12th of August 1976 at the Prinsengracht in Amsterdam. She moved with her family to Tilburg, in the South of the Netherlands, where she attended the Gymnasium at the Theresia Lyceum. She studied Medicine at the University of Amsterdam and did her graduation research on pediatrics at the Tijgerberg Hospital in Capetown, South Africa. During her internships she fell in love with the profession of Gynaecology and Obstetrics.

After obtaining the title of Medical Doctor she went to Jakarta and Bali Indonesia to collaborate in HPV research with Dr. Marjon de Boer, gynaecologist, and initiated by late Professor Dr Lex Peters, gynaecological oncologist in the LUMC. When she returned from Indonesia, Professor Peters had received a grant to set up a cervical cancer screening project in low resource settings. Together with Drs. Fleur Henderson, program director, she realized this project in three areas in Indonesia, which resulted in this thesis.

In 2007 she started her residency training in Gynaecology and Obstetrics at the Groene Hart Ziekenhuis in Gouda (Dr. J.C.M. van Huisseling) and at the Leiden University Medical Center (Prof. Dr. G.G. Kenter en Prof. Dr. J.L. van Lith). In 2014 she obtained the qualification of Gynaecologist and Obstetrician and continued to work at the Groene Hart Ziekenhuis in Gouda. From 2016-2018 she did a fellowship training in Gynaecological Oncology (Professor N.F. Hacker) at the Royal Hospital for Women in Sydney, Australia, resulting in the qualification of Gynaecological Oncologist.
For two years, she then joined the Gynaecological Oncology department in Singleton Hospital in Swansea, Wales, UK (Professor K. Lutchman-Singh). After she enjoyed some time working back in The Netherlands at the Groene Hart Ziekenhuis in Gouda and at the Catharina Ziekenhuis in Eindhoven, she has now returned to Swansea. She has rejoined the Female Cancer Foundation and is involved in cervical cancer screening projects in Bangladesh and Nepal.

Above all this, she married Romek van Thiel and together they have 3 children: Tomas, Olivia and Julius.

Publications

A Performance Evaluation of an Optoelectronic Cervical Screening Device in Comparison to Cytology and HPV DNA Testing
Jessica N Vet, James P. Haindl, Carolina Velasquez, Leonie J. Parker, Margaret I. Burns, Stephen Morrell, Michael J. Campion
European Journal of Gynaecological Oncology 2022 Vol. 43, Issue 2, April 2022 pp213-218

Pelvic Exenteration for the Treatment of Locally Advanced Vulvar Cancer in South West Wales.
Abdulrahman GO, Das N, Chandrasekaran TV, Khot U, Drew PJ, Bose P, **Vet JN**, Tofazzal N, Roberts S, Lutchman Singh K.
Cancers (Basel). 2022 Mar 31;14(7):1767. doi: 10.3390/cancers14071767.

Differences in genetic variation in antigen-processing machinery components and association with cervical carcinoma risk in two Indonesian populations.
Mehta AM, Spaans VM, Mahendra NB, Osse EM, **Vet JN**, Purwoto G, Surya IG, Cornian S, Peters AA, Fleuren GJ, Jordanova ES.
Immunogenetics. 2015 Jun;67(5-6):267-75.

Single-visit approach of cervical cancer screening: see and treat in Indonesia.
Vet JN, Kooijman JL, Henderson FC, Aziz FM, Purwoto G, Susanto H, Surya IG, Budiningsih S, Cornain S, Fleuren GJ, Trimbos JB, Peters AA.
Br J Cancer. 2012 Aug 21;107(5):772-7.

Prevalence of human papillomavirus in Indonesia: a population-based study in three regions.
Vet JN, de Boer MA, van den Akker BE, Siregar B, Lisnawati, Budiningsih S, Tyasmorowati D, Moestikaningsih, Cornain S, Peters AA, Fleuren GJ.
Br J Cancer. 2008 Jul 8;99(1):214-8.

Human papillomavirus type 18 and other risk factors for cervical cancer in Jakarta, Indonesia.
de Boer MA, **Vet JN**, Aziz MF, Cornain S, Purwoto G, van den Akker BE, Dijkman A, Peters AA, Fleuren GJ.
Int J Gynecol Cancer. 2006 Sep-Oct;16(5):1809-14.

Dankwoord

In 2004 ontving Professor Lex Peters een subsidie van de Goede Doelen van de Postcodeloterij om zijn droom waar te maken: een wereld zonder baarmoederhalskanker.

Het voorstel luidde als volgt: “Met mobiele klinieken low resource areas bezoeken om vrouwen te screenen met azijnzuur en deze indien nodig in dezelfde sessie te behandelen met cryotherapie". Effectief, simpel en betaalbaar! Een top-idee, zo vond ook de Postcode loterij en verstrekte 2,1 miljoen euro om dit project te verwezenlijken. Dat was het begin van het See and Treat Project van de Female cancer Program (FcP) in Indonesië en in Suriname.

Gewapend met een enorme hoeveelheid idealisme en krachtige woorden: best available, grassroot level, sustainability, awareness, women empowerment, implementation, teach the teacher, 80% coverage, cost effectiveness en impact, reisden Fleur Henderson en ik af naar Indonesië. We gingen “Memoranda of Understanding” aan met de universiteiten en universitaire ziekenhuizen in Jakarta, Bandung en Den Pasar en ook met de Indonesische Kankerstichting (YKI) en de GGD (PKK). Samen met Indonesische gynaecologische oncologen, pathologen, cytologen en epidemiologen zetten we het screenings project zo op dat het implementeerbaar was in de Indonesische samenleving. In een centrale training in Jakarta trainden we de teams uit de verschillende gebieden om vrouwen op te kunnen roepen, te informeren, te screenen en zo nodig te behandelen. We kochten bussen om de rurale gebieden in te gaan, ontwikkelden informatiefolders en schaften materialen aan om te kunnen screenen en behandelen en om onderzoek te doen naar HPV en afweer. Ook zetten we een verwijzingssysteem naar de Universitaire ziekenhuizen op voor de vrouwen die met baarmoederhalskanker werden gediagnosticeerd.

Na een paar maanden tijd was het pilotproject verwezenlijkt en nadat het van de kinderziekten was genezen, konden we uitbreiden. Uiteindelijk hebben we in de genoemde gebieden 22.000 vrouwen gescreend en zo nodig behandeld. Na dit eerste grote screenings project konden we met een EU-subsidie uitbreiden in Indonesië: van drie gebieden naar acht gebieden. Na een aantal jaren hebben we een belangrijk doel van dit initiatief bereikt: de Indonesische regering nam het screeningsproject op in het landelijke screenings programma.

Wat begon als het FcP, is nu een prachtige Foundation geworden. De Female Cancer Foundation (FCF) heeft een professioneel onbezoldigd bestuur met een projectmanagement team en supervisieartsen. Er zijn in de afgelopen jaren veel nieuwe projecten opgezet waaronder een groot project in Indonesië en momenteel projecten in Bangladesh, Oeganda, Suriname, Nepal en Sierra Leone. Goede samenwerking met bestaande lokale organisaties blijft van het allergrootste belang in

deze projecten. Ondertussen zijn er honderden gezondheidswerkers getraind en honderdduizenden vrouwen voorgelicht, gescreend en zo nodig behandeld. Dat betekent dat duizenden vrouwen daadwerkelijk zijn gered van een potentieel levensbedreigende aandoening.

Ik ben zeer dankbaar dat mijn vader zag dat het aanbod om dit project te gaan opzetten “One in a million” was en dat hij mij aanmoedigde om het avontuur aan te gaan. Het was mij meer dan een waar genoegen hieraan te hebben kunnen bijdragen. Het was inspirerend om omringd te zijn door zoveel enthousiaste, flexibele en gedreven mensen: alle lof voor het hele team in Indonesië en alle lof voor het pathologie lab in het LUMC.

Zeer veel dank gaat uit naar degenen met the everlasting support, positieve en kritische blik: prof. GertJan Fleuren, prof. Baptist Trimbos, prof. Vincent Smit, dr. Marjon de Boer, drs. Fleur Henderson, dr. Gatot Purwoto, dr. Annemiek Doeksen, dr. Hans van Huisseling en prof. Neville Hacker. En prof. Lex Peters! Lex: je bent niet meer onder ons, maar je missie wordt voortgezet.

Mijn bijzondere dank en veel liefde voor degenen die er waren in every step of the way: Romek, Ireen, Julia en Carolijn. En André, niet uit ons hoofd, nooit uit ons hart ♥.