DEPARTMENT OF WOMEN'S AND CHILDREN'S HEALTH
Karolinska Institutet, Stockholm, Sweden

SUGGESTED NEXT STEPS TO PREVENT CERVICAL CANCER AFTER SURGICAL TREATMENT FOR HIGH-GRADE CERVICAL DYSPLASIA

David Megyessi

Karolinska Institutet

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Suggested next steps to prevent cervical cancer after surgical treatment for high-grade cervical dysplasia

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By

David Megyessi

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Principal Supervisor:
Associate Professor Miriam Mints
Karolinska Institutet
Department of Department of Women's and Children's Health
Division of Obstetrics and Gynecology

Co-supervisor(s):
M.P.H., Ph.D. Ellinor Östensson
Karolinska Institutet
Department of Department of Women's and Children's Health
Division of Obstetrics and Gynecology

M.D., Ph.D. Susanna Alder
Karolinska Institutet
Department of Department of Women's and Children's Health
Division of Obstetrics and Gynecology

Opponent:
Professor Tina Dalianis
Karolinska Institutet
Department of Oncology-Pathology

Examination Board:
Associate Professor Arne Wikström,
Karolinska Institutet
Department of Medicine
Division of Dermatology and Venereology

Associate Professor Ilona Lewensohn-Fuchs
Karolinska Institutet
Department of Laboratory Medicine
Division of Clinical Microbiology

Associate Professor Gisela Helenius,
Örebro Universitet
School of Medical Sciences

In Memory of Prof. Sonia Andersson

It is with a heavy heart that I mourn the loss of my main supervisor, Sonia Andersson. She was more than just a supervisor to me, she was a guide, a friend and an **extra mother**. She was always there to offer support, advice, and encouragement, and she played a significant role in shaping my doctoral project.

I will remember the countless conversations we had and the lessons she taught me. She never stopped guiding me, even from her hospital bed, she continued to plan and discuss future projects – a true researcher! She will be greatly missed by all who knew her – family, friends, colleagues, researchers and the thousands of patients she has helped through her research and as a gynecologist.

While working on this thesis, Sonia's presence continues to be felt in my life and I often imagine her by my side, still guiding me and offering words of wisdom. Although she may be gone, her legacy will live on through the many lives she touched.

Rest in peace, Sonia. You will never be forgotten.

Sincerely,

David Megyessi

Popular science summary of the thesis

Cervical cancer is a highly preventable disease through vaccination, screening and treatment. However, it is still the fourth most common cancer among women worldwide and every two minutes, a life is lost to the disease. Because of global inequality, the majority of all cervical cancer cases occur in low- and middle-income countries. There are two major types of cervical cancer that arise from the two types of cells that line the uterus: squamous cell carcinoma of the cervix and adenocarcinoma. Cervical squamous cell carcinoma is more common, accounting for 80% of all cervical cancers. Nearly all cases of cervical cancer are caused by human papilloma virus (HPV) infection, which is the most common sexually transmitted infection in the world. Additionally, infection with HPV can also cause oropharyngeal, vaginal, vulvar, penile and anal cancers. There are more than 220 HPV genotypes, at least twelve of which are classified as high-risk HPV because they cause cancer. Approximately 70% of all cervical cancers are caused by high-risk HPV 16 and 18. Approximately 90% of all HPV infections are cleared by the immune system within two years and only a minority develop into cancer. In 2006, the first HPV vaccine became available and since then, several HPV vaccines have developed. In 2014, Gardasil-9 was launched, which protects against two low-risk HPV types (causing genital warts) and seven high-risk HPV types. Over 90% of all cervical cancers can be prevented by Gardasil-9.

In Sweden, the incidence of cervical cancer and associated mortality rates have dramatically decreased since the introduction of national cervical cancer screening programs in the 1960s. However, over the past decade, this decrease has stagnated and instead an increase in cervical cancer is again being observed.

Women who do not attend screening and women who have received treatment for precancerous lesions are the two principial risk groups of developing cervical cancer in Sweden. The latter group are at greater risk of developing cervical cancer than the general population. This risk remains elevated for more than two decades. Therefore, an optimal follow-up strategy after treatment is crucial to protect these women from cervical cancer. Identification of predictors of treatment failure will help to optimize follow-up surveillance post-treatment and to guide retreatment in relevant cases. These are the main aims of this thesis.

Studies 1, 2 and 3 followed women who had previously been treated for high-grade cervical dysplasia to help assess risk of recurrence. In conclusion, results show that HPV status, subdivision of margin involvement into ecto- and endocervical cell types, age, comorbidity and smoking status were all found to be useful predictors of treatment failure. Individualized follow-up guided by these risk factors is imperative in the effort to attenuate the increasing incidence of cervical cancer in Sweden.

Populärvetenskaplig sammanfattning

Livmoderhalscancer är en sjukdom som går att förebygga genom vaccination, screening och behandling. Trots detta är det fortfarande världens fjärde vanligaste cancersjukdom bland kvinnor. Varje minut dör två människor till följd av sjukdomen. På grund av globala orättvisor sker majoriteten av fallen i låg- och medelinkomstländer. Det finns två huvudsakliga typer av livmoderhalscancer som utgår från de två celltyper som täcker livmoderhalsen; skivepitelcancer och körtelcellscancer. Den vanligaste förekommande typen är skivepitelcancer som utgör ca 80% av alla fall. Nästan all livmoderhalscancer orsakas av humant papillomvirus (HPV) som är världens vanligaste sexuellt överförbara infektion. Utöver livmoderhalscancer kan HPV-infektionen leda till cancer i munhåla/svalg, slida, vulva, penis och ändtarm. Det finns över 220 olika typer av HPV varav åtminstone tolv är klassade som "hög-risk HPV", vilka är de HPV-typer som orsakar cancer. Omkring 70% av alla fall av livmoderhalscancer orsakas av HPV-typerna 16 och 18. Uppemot 90% av alla HPV-infektioner läker ut spontant inom två år och endast en liten bråkdel kvarstår och utvecklas till cancer. Det första HPV-vaccinet kom 2006 och idag finns flera HPV-vacciner tillgängliga. Gardasil-9 lanserades 2014 och skyddar mot två "låg-risk HPV"-typer (som orsakar könsvårtor) och sju "hög-risk HPV"-typer. Detta vaccin ger över 90% skydd mot livmoderhalscancer.

Sedan screeningen för livmoderhalscancer introducerades i Sverige på 60-talet har förekomsten och dödligheten i sjukdomen avsevärt minskat. Under det senaste decenniet har denna sjunkande trend dock avstannat och istället har man åter observerat en ökning av antalet nya fall av livmoderhalscancer.

Kvinnor som inte deltar i screeningen samt kvinnor som tidigare erhållit behandling mot cellförändringar i livmoderhalsen är de grupper som i huvudsak riskerar att utveckla livmoderhalscancer i Sverige. Den senare gruppen löper ökad risk att utveckla livmoderhalscancer jämfört med normalbefolkningen. Riskökningen kvarstår i mer än två decennier. Optimal uppföljning efter behandling är därför av avgörande vikt för att skydda dessa kvinnor från livmoderhalscancer. Huvudsyftet med denna avhandling är att bidra till förebyggandet av livmoderhalscancer efter behandling av cellförändringar genom att identifiera faktorer som kan förutspå behandlingssvikt. Därmed kan uppföljningen förbättras och ge vägledning till vilka kvinnor som behöver behandlas på nytt.

I studie 1, 2 och 3 har kvinnor som erhållit behandling mot höggradiga cellförändringar i livmoderhalsen följts upp och risken för återfall utvärderats. Sammanfattningsvis talar resultaten för att HPV-status, uppdelad utvärdering av operationsmaterialets resektionsränder, ålder, samt att väga in samsjuklighet och rökning, alla är faktorer som kan användas för att göra en riskbedömning om behandlingssvikt föreligger. Förhoppningsvis kan detta bidra till att dämpa den ökade incidensen av livmoderhalscancer i Sverige.

Abstract

The risk of cervical cancer among women treated for high-grade cervical dysplasia is more than twofold compared with the general population, and this risk remains elevated for over two decades. In Sweden, cervical cancer incidence is rising again and the risk of cervical cancer among women with a prior history of high-grade cervical dysplasia has increased since the 1960s. The surgical procedure known as conization is commonly used to treat high-grade cervical dysplasia and prevent progression to invasive cervical cancer. However, treatment failure, defined as residual/recurrent/ high-grade cervical dysplasia or cervical cancer post-conization, has reportedly increased by almost twenty percent. Suggested risk-factors for post-conization treatment failure include age, smoking, treatment modality, lesion size and severity, incomplete excision of lesion, infection with high-risk human papilloma virus (hrHPV) and hrHPV persistence. The overarching aim of this thesis addresses how to protect women from developing cervical cancer following treatment of high-grade cervical dysplasia. The included studies examine risk factors for recurrent disease and what factors or combinations thereof can accurately predict treatment failure and thereby identify women at high risk post-conization.

Study I investigated the long-term risk of residual/recurrent high-grade cervical dysplasia post-conization and how such risk varies according to margin status, comorbidity and HPV infection. The study included a total of 991 women who had undergone conization for high-grade cervical dysplasia between 2000 and 2007. Data were obtained from medical records and the Swedish National Cervical Screening Registry (NKCx). Given a median follow-up of ten years and maximum of sixteen years, almost twelve percent of the cohort was diagnosed with residual/recurrent disease or worse (invasive cervical cancer). A greater than 2.5-fold risk of recurrent disease was found among women with incomplete resection compared with cases where the margins were clear. Risk varied according to the extent of anatomical infiltration of disease margins and was particularly elevated when endocervical margins were positive. Comorbidities such as autoimmune disease, HIV, hepatitis B and/or C, malignancy, diabetes, and genetic disorder and/or organ transplantation were independent predictors of recurrent disease. For the subgroup of women who were hrHPV positive with involved margins, risk of recurrent disease was increased compared with the subgroup of women who were HPV positive with clear margins. Women with incompletely resected precancerous lesions are at increased risk for recurrent/residual high-grade cervical dysplasia and cervical cancer. Combined assessment of margin and hrHPV status, while also taking comorbidities into account, may provide a useful strategy to accurately identify at-risk women who should undergo reconization.

Study II evaluated risk of recurrent disease among women who had undergone first-time treatment for high-grade cervical dysplasia, within a cohort where complete HPV status

was known. A total of 529 women were included, all of whom had undergone conization for high-grade cervical dysplasia between 2014 and 2017. Follow-up continued for up to six years post-conization, during which time 22 patients were diagnosed with recurrence of high-grade cervical dysplasia. Four significant independent risk factors for recurrence were identified: age 45 or older, involved margins, positive hrHPV test at first follow-up and abnormal cytology at first follow-up. Furthermore, persistent hrHPV infection was associated with recurrent disease. The finding that involved margins are an independent risk factor suggests that more intense follow-up is required for these women, regardless of early HPV status post-conization. Although early HPV-positive status post-treatment was found to be a strong independent risk factor for predicting recurrent disease, more than 30% of the 22 patients diagnosed with recurrent disease were HPV-negative shortly after treatment. These patients, however, were subsequently found to be HPV-positive on routine screening, suggesting that repeated HPV testing is necessary during post-conization follow-up.

Study III explored risk factors for recurrent/persistent adenocarcinoma-in-situ (AIS), as well as risk factors for progression from AIS to invasive cervical cancer among women who had previously undergone conization for AIS. A total of 84 women who had primary treatment with conization for AIS between 2001 and 2017 were included. Twelve women developed recurrent disease, two of whom had invasive cervical cancer. Among all factors, one or more positive HR-HPV assays post-conization provided the highest sensitivity for predicting recurrence, while smoking or past history of smoking were associated with the highest specificity for recurrence. When adjusting for age at conization and abnormal cytology at follow-up, we demonstrated that HPV18 positive status was the strongest predictor for post-conization recurrence. Two or more positive HPV results post-conization helped predict recurrence. The strong predictive value of HPV in relation to recurrence, especially HPV18, indicates that HPV testing during post-treatment follow-up for AIS is necessary. In addition, it is important to consider smoking status and to encourage long-term follow-up so as to better protect these women who are at high risk of recurrence and progression to invasive cervical cancer.

In conclusion, this thesis improves our understanding of what risk factors are able to accurately predict treatment failure and how to identify women at risk of recurrent disease after treatment. This thesis highlights the importance of individualized long-term follow-up, including evaluation of margin status based on residual tumor classification, the need for repeated HPV testing during follow-up and attention to comorbidities.

List of scientific papers

I. Alder S, MEGYESSI D, Sundström K, Östensson E, Mints M, Belkić K, Arbyn M, Andersson S.
Incomplete excision of cervical intraepithelial neoplasia as a predictor of the risk of recurrent disease–a 16-year follow-up study
Am J Obstet Gynecol. 2020 Feb;222(2):172.e1–172.e12. doi: 10.1016/j.ajog.2019.08.042. Epub 2019 Aug 29. PMID: 31473226.

II. Andersson S*, MEGYESSI D*, Belkić K, Alder S, Östensson E, Mints M.
Age, margin status, high-risk human papillomavirus and cytology independently predict recurrent high-grade cervical intraepithelial neoplasia up to 6 years after treatment
Oncol Lett. 2021 Sep;22(3):684. doi: 10.3892/ol.2021.12945. Epub 2021 Jul 27. PMID: 34434283; PMCID: PMC8335741.
*Contributed equally

III. Belkić K, Andersson S, Alder S, Mints M, MEGYESSI D.
Predictors of treatment failure for adenocarcinoma in situ of the uterine cervix: Up to 14 years of recorded follow-up
Oncol Lett. 2022 Aug 25;24(4):357. doi: 10.3892/ol.2022.13477. PMID: 36168314; PMCID: PMC9478621.

Contents

List of abbreviations

ACIP	The Advisory Committee on Immunization Practice
ADC	Adenocarcinoma
AGC–FN	Atypical glandular cells favoring neoplasia
AGC–NOS	Atypical glandular cells not otherwise specified
AIS	Adenocarcinoma in situ
AJCC	The American Joint Committee on Cancer
ASC–H	Atypical squamous cells cannot exclude HSIL
ASC–US	Atypical squamous cells of undetermined significance
C–LETZ	Contoured–loop excision of the transformation zone
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CIN2+	CIN2+ or worse
CT	Chlamydia trachomatis
EMA	European Medicine Agency
FIGO	Federation of Gynecology and Obstetrics
HC1	Hybrid Capture first genereation
HC2	Hybrid Capture second generation
HIV	Human immunodeficiency virus
HPV	Human papilloma virus
HR	Hazard ratio
hrHPV	High-risk human papilloma virus
HSIL	High-grade intraepithelial lesions
HSV–2	Herpes simplex virus type 2
IARC	The International Agency for Research on Cancer
ICD	International Classifications of Diseases
KUH	Karolinska University Hospital
LBC	Liquid based cytology
LCR	Long control region
LEEP	Loop electrosurgical excision procedure

lrHPV	Low-risk human papilloma virus
LSIL	Low-grade intraepithelial lesions
MDT	Multi-disciplinary team
MLR	Multiple logistic regression
MSM	Men who have sex with men
MW	Mann-Whitney test
NILM	Negative for intraepithelial lesions or malignancy
NKCx	the Swedish National Cervical Screening Registry
NOMESCO	Nordic Medico-Statistical Committee
NPV	Negative predictive value
OC	Oral contraceptive
OR	Odds ratio
Pap smear	Papanicolaou smear
PCR	Polymerase chain reaction
PPV	Positive predictive values
RO	the Basic Reproductive Number
Rb	Retinoblastoma
SCC	Squamous cell carcinoma
SCJ	Squamocolumnar junction
ssHPV	Self-sampled human papilloma virus test
STI	Sexual transmitted infection
TBS	The Bethesda System
TNM	The tumor, node and metastasis system
TZ	Transformation zone
US FDA	The US Food and Drug Administration
VSS	Self-collected vaginal samples
WHO	The World Health Organization

1 Introduction

In 2008, Harald zur Hausen was awarded the Noble prize "for his discovery of human papilloma viruses (HPV) causing cervical cancer." This discovery has led to improved cervical cancer prevention via HPV vaccination, HPV-based cervical screening and HPV testing as a test of cure after treatment of precancerous cervical lesions. Despite these scientific breakthroughs, and even though cervical cancer is highly preventable, this disease is still the fourth most common cancer among women worldwide.

The global distribution of cervical cancer is skewed. More than 90% of cases occur in low- and middle-income countries, mainly because of inadequate access to vaccination, screening and treatment. In 2020, the World Health Organization (WHO) approved a strategy for the elimination of cervical cancer within a generation. The WHO aims to achieve its "90:70:90" targets by 2030, according to which 90% of girls are HPV vaccinated, 70% of women undergo screening and 90% of women identified with cervical disease are treated.

In Sweden, thanks to HPV vaccination, national cervical screening programs and subsequent treatment of precancerous lesions, the incidence of cervical cancer and associated mortality rates have decreased dramatically since the 1960s. However, cervical cancer incidence is rising once again. A national reexamination was performed on normal cytology prior to detected cervical cancer between 2008-2016. Approximately 30% of the results were false negative.

There are two main risk groups of developing cervical cancer in Sweden: women who do not attend screening and women who have previously received treatment for precancerous lesions. For the latter group, the risk of developing cervical cancer is more than twofold compared with the general population, and this risk remains elevated for more than 25 years after treatment.

This thesis focuses on those women who have received treatment for high-grade cervical dysplasia. Cervical dysplasia will be thoroughly reviewed, including a general background on the anatomy of the cervix, cervical cancer epidemiology and risk factors, as well as HPV facts, prevention and treatment options. The sections included in this thesis are research aims, methods, results, discussions and conclusions. Lastly, points of perspective are discussed.

The overarching goal is to contribute to cervical cancer prevention through identification of predictors of treatment failure in order to optimize follow-up surveillance post-treatment so as to protect these high-risk women from developing cervical cancer.

2 Background

2.1 The Cervix

The cervix is the lower part of the uterus, which connects to the vagina through the endocervical canal. The outer part of the cervix is known as the ectocervix, which is contiguous with the vaginal wall and covered by stratified squamous epithelium. The endocervix is the inner part of the cervix that leads to the uterus through the endocervical canal and is covered with columnar epithelium. The squamocolumnar junction (SCJ) is the anatomical region where the stratified squamous epithelium and the columnar epithelium meet. The SCJ is dynamic and undergoes changes throughout life. During puberty the SCJ migrates from the endocervical canal toward the ectocervix, becoming more visible on the surface of the portio, or cervical lip. The epithelium between the new and the original SCJ is called the transformation zone (TZ). Postmenopause, the SCJ usually migrates back into the endocervical canal and becomes less visible once again. Premalignant transformation often occurs in the TZ (1, 2). Squamous cell carcinoma (SCC) and the considerably less common adenocarcinoma (ADC) are the two main types of cervical cancer that arise from the two types of cervical epithelium (3).
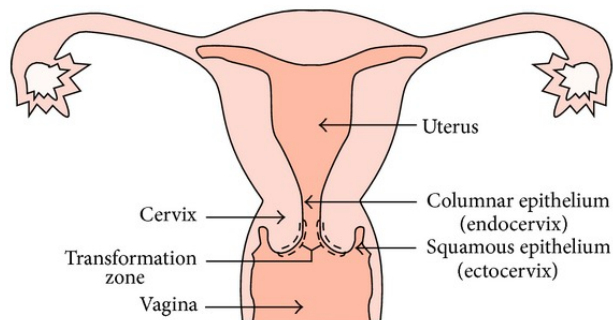
Figure 1. Anatomy of a uterus showing endocervix, ectocervix and the TZ. Adapted from Bengtsson et al. (4) under Creative Commons Attribution License (CC BY).

2.2 Cervical cancer epidemiology

Cervical cancer is the fourth most common cancer among women worldwide, accounting for 6.9% of all female cancers. In 2020, approximately 600 000 cases of cervical cancer and 34 000 deaths were registered. The overall age-standardized incidence rate is 13.3 per 100 000 women-years, while the mortality rate is 7.3 per 100 000 women-years. Incidence rates vary among countries, ranging from 2 to 84 per 100 000 women, while mortality rates range from 1 to 56 per 100 000 women (5). However, the burden of cervical cancer is unevenly distributed and the incidence and mortality rates vary significantly by

geographical region. The majority of cases occur in low- and middle- income countries, which account for more than 85% of deaths worldwide from cervical cancer (6-8).

ASR (World) per 100 000
- ≥ 25.2
- 16.7–25.2
- 11.7–16.7
- 7.0–11.7
- < 7.0
- Not applicable
- No data
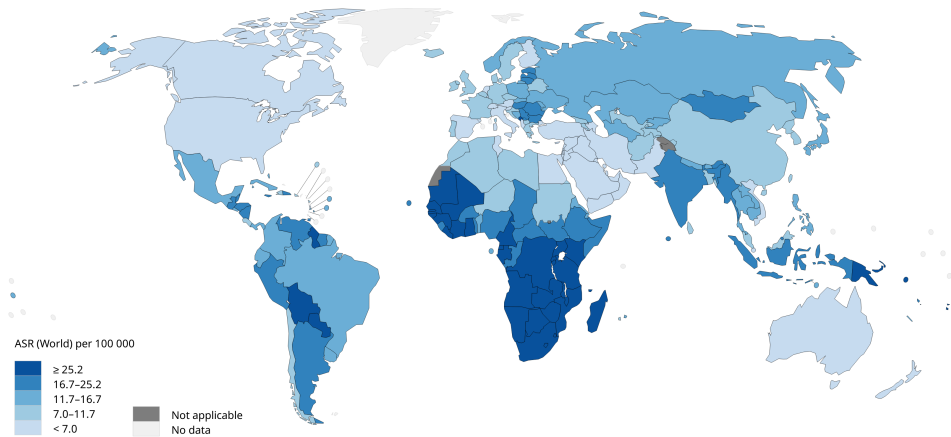
Figure 2. World map of the estimated age-standardized incidence rates (per 100,000) of cervical cancer cases in 2020. Data source: GLOBOCAN 2020 Map production: IARC (http://gco.iarc.fr/today) World Health Organization.

2.3   Human papilloma virus (HPV)

Human papilloma virus (HPV) is the main etiological factor in almost all cases of cervical cancer (1, 6, 9–11). Viruses of the papilloma family are small, non-enveloped, double-stranded circular DNA viruses, infecting the epithelium of the skin and the mucosa.

Figure 3. Genomic organization of the HPV genome. Schematic representation of the HPV16. Adapted from D'Abramo CM et al. (12) under Creative Commons Attribution Non-Commercial License (CC BY-NC).

2.3.1   Classification, taxonomy and structure

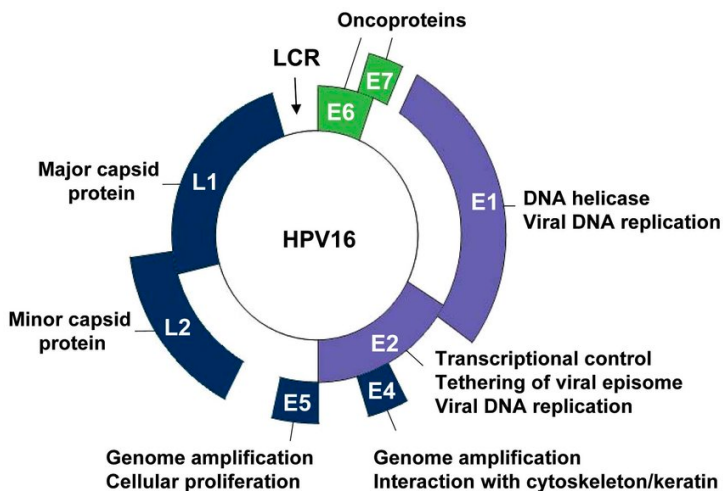There are more than 220 types of HPV, of which approximately 50 are capable of infecting the genital tract. Some HPVs are frequently detected in cancers, while others are rarely found, which has led to the nomenclature of "high-risk HPV" (hrHPV) and "low-risk HPV" (lrHPV). The International Agency for Research on Cancer (IARC) has classified twelve HPV types as carcinogenic to humans (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) and one HPV type (68) as probably carcinogenic to humans. These thirteen hrHPV types are linked with malignant mucosal infections. Due to more recent studies done, HPV 66 has been re-classified and is not considered to be carcinogenic to humans. Low-risk HPVs are non-carcinogenic and include types such as HPV 6 and 11, which cause condyloma (genital warts) (13, 14).

HPV 16 and 18 contribute to 70% of all cervical cancers (1, 15) and 50% of all cervical intraepithelial neoplasia grade 3 (CIN3) (1). Several studies have shown that HPV 16 is more frequently found in SCCs and HPV 18 in ADCs (16–18).

HPV is known to mainly infect the cutis, as well as the oral, anal and genital squamous epithelium, including the endocervical columnar epithelium (19). There are five evolutionary groups of HPVs (Alpha, Beta, Gamma, Mu and Nu) based on their epithelial tropism and what diseases they cause. The alpha group can cause cutaneous and mucosal infections, while the other groups only cause cutaneous infections. All thirteen hrHPVs and lrHPVs mentioned above belong to the alpha group (20).

The HPV genome is organized into three main regions: 1) early genes (E1, E2, E3, E4, E5 and E7) encode several proteins important for viral replication, transcription and cell transformation; 2) late genes (L1 and L2) encode proteins necessary for assembly of the viral capsid, and 3) the long control region (LCR) is involved in viral DNA replication and transcription. The E6 and E7 oncoproteins are considered to be the major oncogenes, the function and properties of which account for the main difference between hrHPV and lrHPV. Overall, the E6 and E7 oncoproteins of hrHPV have greater oncogenic transformation potential than their equivalents in lrHPV due to their more effective inactivation and degradation of tumor suppressor protein p53 and Retinoblastoma (Rb) (20, 21). Cell cycle entry is regulated by Rb and the interaction between E7 and Rb induces abnormal cell proliferation. Cell cycle arrest, DNA repair and apoptosis induction are regulated by p53 and by binding to it, E6 blocks apoptosis and promotes cell proliferation (22).

2.3.2   Viral life cycle

HPV enters the epithelium in the cervical TZ through microabrasions and small wounds. HPV gains access to the basal layer of the epithelium, where its genome maintains a low

copy number and viral gene expression is restricted (23, 24). Infected cells of the basal layer undergo cell division and new infected daughter cells migrate upwards to the surface of the epithelium. Once these cells reach the upper part of the epithelium, differentiation into mature epithelial cells occurs and normal cell division stops. At the same time, all viral genes become activated. Replication results in high copy numbers of the viral genome and late capsid genes are synthesized, resulting in release of virions from the outermost layer of the epithelium with the potential to spread the virus to others (23–25).
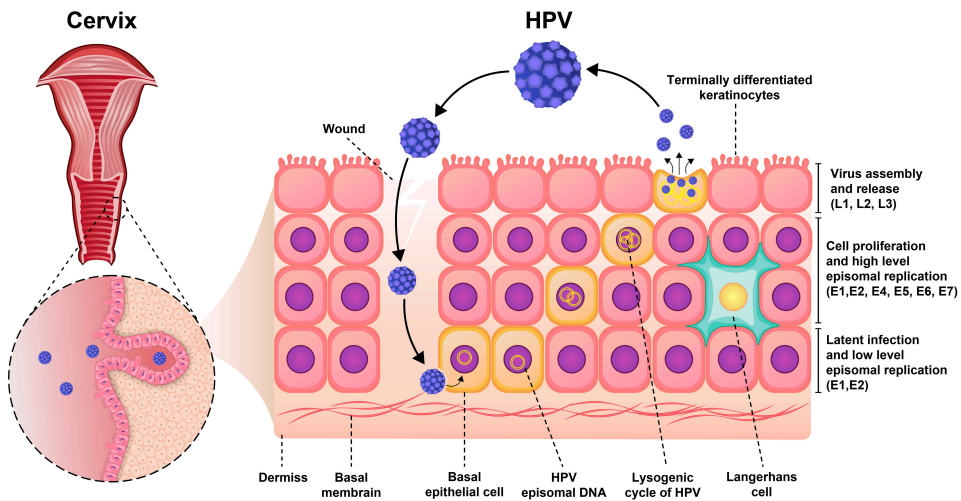

Figure 4. Pathogenesis of HPV infection. Adapted from Yousefi Z et al. (26), under Creative Commons Attribution License (CC BY).

2.3.3   Immune response

HPV infection is characterized by intraepithelial infection which effectively hides the virus from the host immune system without causing viremia, inflammation, or apoptosis. Nevertheless, most HPV infections are transient and the immune system clears up to 90% of all HPV infections within two years. The underlying mechanisms of the immune response have not yet been fully elucidated, although both humoral and cell-mediated components are involved. Neutralizing antibodies play a key role in preventing initial viral entry and cellular immunity is thought to be primarily responsible for viral clearance (27). Still, a minority of HPV infections are not effectively cleared by the host immune system and may persist and become chronic. Persistent hrHPV is associated with high-grade dysplasia, which if left untreated, may result in invasive cervical cancer (20, 28). However, the definition of persistent HPV infection may vary. Though commonly defined as having two or more positive HPV tests, some base the definition on time-to-clearance or proportion of HPV-positive visits. The definition is further complicated by differences in

analytical methodology and frequency of testing (29). According to the Swedish national guidelines, persistent HPV is defined as testing positive for HPV 16, 18 and/or 45 in two samples at least twelve months apart, or testing positive for HPV 31, 33, 52 and/or 58 in two samples at least 30 months apart, or as testing positive for HPV 35, 39, 51, 56, 59, 66 and/or 68 in two samples at least 54 months apart (30).
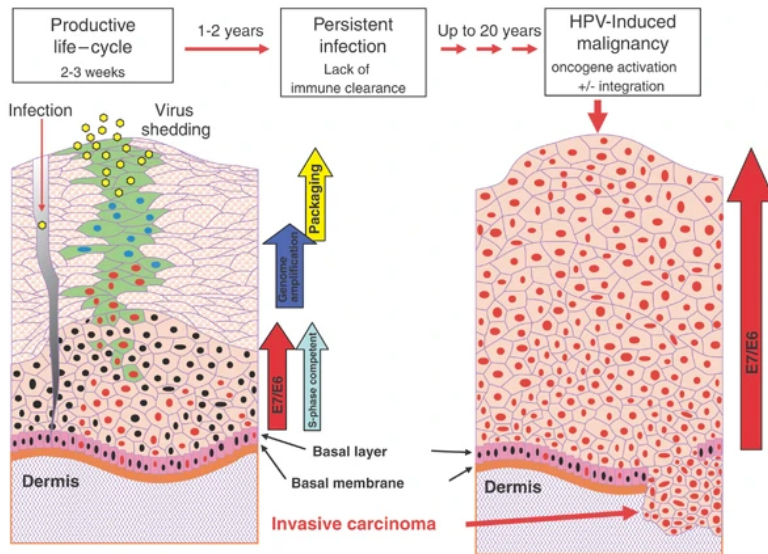
Figure 5. Progression from persistent HPV infection to malignancy. Reproduced with kind permission from Thomas et al. (license number 5530710217668) (31)

2.3.4 Methods for detection of HPV

The laboratory uses HPV assays to detect the presence of HPV in clinical samples. Many HPV tests are available on the market, but only clinically validated tests should be used (32). Currently, there are more than 250 commercial tests with different properties that can be divided into different groups based on their ability to distinguish between different HPV types, a process known as genotyping. DNA testing is capable of full genotyping into individual types of hrHPV in a single reaction, while HPV DNA testing with partial genotyping usually provides results that group various hrHPV types together, e.g., positive for HPV16/18/45 (33, 34). HPV assays used in the screening program usually cover fourteen hrHPV types, including HPV 66 (which is not anymore classified as oncogenic to humans) (30).

2.3.4.1 Hybrid capture 2

Hybrid capture 2 (HC2) was approved by the US Food and Drug Administration (US FDA) in 1999 when it replaced the original Hybrid capture (HC1). HC2 has been clinically validated for use in cervical cancer screening. This test is based on hybridization of HPV DNA and HPV RNA. Specific antibodies are used to label hybridization between the RNA probe and matching DNA, which can then be detected through chemiluminescence. HC2 can detect thirteen hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and five lrHPV types (6, 11, 42, 43, 44). The test results are given as either hrHPV–positive or hrHPV–negative, but cannot identify specific HPV types (35, 36).

2.3.4.2 Polymerase chain reaction

A specific DNA fragment can be amplified using polymerase chain reaction (PCR) to produce millions of copies of the gene. PCR technique is extremely sensitive, with a detection threshold of just one viral copy. Using different primers, often targeting the L1 region, it is possible to carry out HPV genotyping. One example is the GP5+/bio–GP6+ which uses primers that amplify the L1 region. It is clinically validated and can detect 14 types of hrHPV and 23 types of lrHPV. This technique is used in research, but it is not commercially available, nor is it used for cervical cancer screening.

The clinically validated Cobas HPV test is a real–time PCR assay capable of detecting fourteen hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). The fully automated system targets the hrHPV L1 gene and uses the β–globin gene as an internal control. The Abbot RealTime hrHPV assay detects fourteen hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). The BD HPV Assay is clinically validated and amplifies hrHPV E6/E7. It detects fourteen types of hrHPV and provides individual results for six HPV types (16, 18, 31, 45, 51, and 52). The Xpert HPV detects hrHPV encoding for fourteen hrHPV types. The APTIMA HPV assay is an example of an HPV test to detect E6/E7 mRNA. It is capable of detecting fourteen types of hrHPV through real time amplification (35).

2.3.5 Prevalence and transmission

Hundreds of millions of people are infected by HPV, making it one of the most common virus infections and the most common sexually transmitted infection (STI) worldwide. Incubation time may vary from weeks to months, and even to years, likely depending on viral dose (37). HPV prevalence among women with normal cytology is approximately 10% and highest in developing countries, in some regions as high as 30%. HPV prevalence is similar between men and women (38, 39). Although many infections are asymptomatic, HPV is more likely to cause disease in women, making the burden higher among women

than in men (38, 40). According to a Swedish study, mean HPV prevalence among women aged 30-64 is approximately 9% (41). Lifetime risk for contracting HPV infection is approximately 80%, however these studies were performed before HPV vaccination (15).

Of all cancers worldwide, the annual incidence of HPV-related cancers is 4.5%, the most common of which by far is cervical cancer. By gender, HPV causes 8.6% of all cancers in women and 0.8% of all cancers in men (42). HPV does not only cause cervical cancer, but plays an etiological role in other cancers as well. While the numbers may vary, it is estimated that HPV is associated with approximately 90% of all anal cancer, 80% of all vaginal cancer, 50% of all penile cancer, 30% of all oral and oropharyngeal cancer and 25% of all vulvar cancer (43).

2.4 Precancerous lesions of the cervix

The cervical intraepithelial neoplasia (CIN) nomenclature was introduced in the 1960s. Cytological characteristics and histological features of different cervical squamous epithelium abnormalities were described in detail (44). Cervical intraepithelial neoplasia is graded into three groups based on severity of dysplasia: mild dysplasia (CIN1), involving the lowest one-third of the epithelium, moderate dysplasia (CIN2), involving two-thirds of the epithelium and severe dysplasia (CIN3), involving more than two-thirds of the epithelium, which is equivalent to carcinoma in situ. Invasive cervical cancer is defined as abnormal cells penetrating through the basement membrane with invasion of underlying tissue (1, 10).

The Bethesda System (TBS) was introduced in the late 1980s with the aim of developing a clinically relevant classification system with higher reproducibility. Two groups were introduced for HPV-related squamous intraepithelial lesions: low-grade (LSIL) and high-grade (HSIL). Since then, TBS has been revised and updated three times (1991, 2001 and 2014) (45). LSIL is equal to mild dysplasia (CIN1) and HSIL corresponds to moderate and severe dysplasia (CIN2 and CIN3 grouped together) (1, 10). Atypical squamous cells are either classified as being of undetermined significance (ASC-US) or as cannot exclude HSIL (ASC-H).

Precancerous lesions of the glandular cells are called adenocarcinoma-in-situ (AIS), first described by Helper in 1952. The glandular epithelium is composed of a single layer of cells (2). Unlike assessment of squamous cell carcinoma, there is no gradation of adenocarcinoma precursors (46). TBS subclassifies atypical glandular cells into either atypical glandular cells not otherwise specified (AGC-NOS) or atypical glandular cells favoring neoplasia (AGC-FN) (47).

Sweden transitioned from the CIN classification system to TBS in early 2017. However, CIN classification is still used for women younger than age 30 who have been diagnosed with HSIL, which is then sub-classified into CIN2 or CIN3, since disease management is different for this age group. CIN2 usually resolves spontaneously, for which reason watchful waiting is a useful strategy to prevent unnecessary treatment (30).
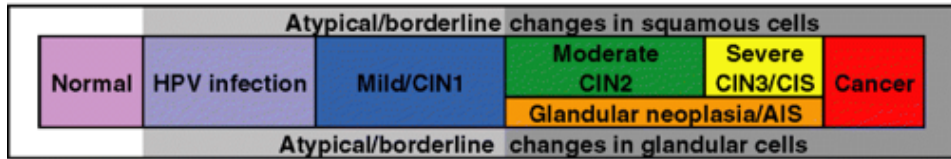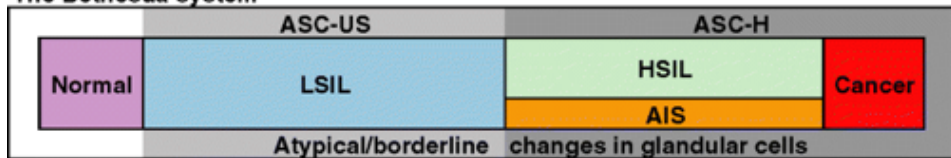
Figure 6. Conceptual categorization of cytological findings. Reproduced with kind permission from Herbert et al. (License number 5530680448611) (48).

2.4.1 Natural history of cervical neoplasia

Both LSIL and HSIL are more likely to regress than progress. The natural history of LSIL is spontaneous regression in the majority of cases (49). Untreated, LSIL will regress within 2–5 years in up to 80% of all cases. CIN2 will spontaneously regress in at least 40% of all cases and only 20% of CIN2 will develop into CIN3, of which less than 5% progress to invasive cancer (50).
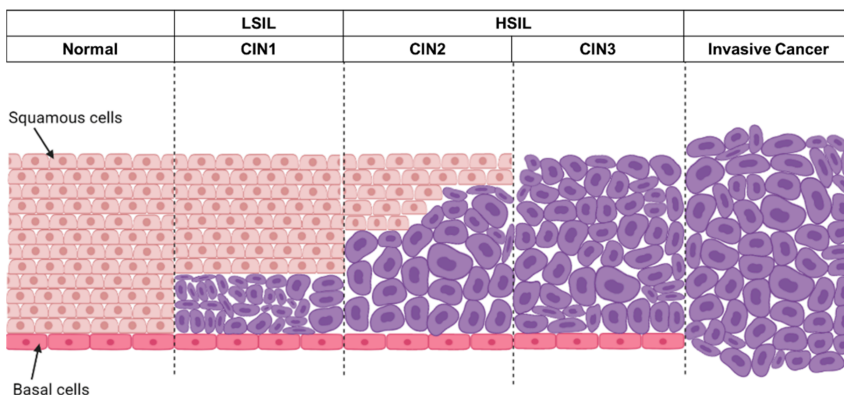
Figure 7. An overview of the progression of squamous cervical cancer. Adapted from Boon et al. (51), under Creative Commons Attribution License (CC BY).

In general, and for understandable reasons, there are few prospective studies evaluating the risk of progression from low to high-grade cervical dysplasia and on to invasive cervical cancer. Various numbers have been reported for rates of regression and progression. One study reported the risk of progression from LSIL to HSIL is 6% within one year and 12% within three years with no cases of invasive cancer (52). Another study showed that LSIL develops into HSIL in 10% of cases within one year and found no cases of invasive cancer (53). According to a Canadian study, the risk of progression from CIN1 to CIN3 or to invasive cancer is 1% per year. The risk of progression from CIN2 to CIN3 was 16% and 25% within 2 and 5 years, respectively (54). A large 2021 meta-analysis that included 89 studies found that most cases of CIN1 and CIN2 spontaneously regress within two years. Progression to invasive cervical cancer was observed in less than 0.5% of cases. Left untreated, CIN1 demonstrates overall regression, persistence and progression to CIN2+ or CIN3+ in 60%, 25%, 11%, and 2% of cases, respectively. Corresponding figures for CIN2 were 55%, 23% and 19%, respectively. For CIN3 these figures were 28%, 67% and 2%, respectively. Regression rates were higher among women younger than age 30 and among hrHPV negative women (55).

Clearly, it would be unethical to leave CIN3 untreated in order to study risk of progression from CIN3 to invasive cervical cancer. Nevertheless, in New Zealand between 1965 and 1974, such an unethical clinical study was conducted in which women with CIN3 did not receive further treatment. Based on the data from that study, McCredie et al. evaluated long-term risk of invasive cancer among patients with untreated CIN3. The cumulative incidence of cervical or vaginal cancer was approximately 30% within 30 years, but rose to more than 50% among women with persistent CIN3 after two years. For women who received appropriate treatment, the cumulative incidence of cervical or vaginal cancer was less than 1% within 30 years (56).

The natural history of cervical glandular dysplasia is less well-defined than for squamous dysplasia, probably because the former is less common. AIS usually arises from the SCJ of the TZ (57). No studies have investigated AIS regression rate, AIS progression rate to invasive cervical cancer, or duration before malignant transformation occurs (58). Few studies report separate outcomes for AIS (59). The estimated duration of AIS before progression to invasive adenocarcinoma is 5-13 years (60). However, the AIS incidence rate has increased in recent decades and currently is 6.6 per 100 000 individuals globally. Average age at time of diagnosis is 35-37 years (61) and more than 50% of women with AIS have concurrent squamous dysplasia (57).

Without therapy, approximately 1% of mild dysplastic lesions (CIN1) and 12% of severe dysplastic lesions (CIN3) will progress to invasive cervical cancer (15). However, progression from precancerous lesions to invasive cancer may take many months to many years (1, 15).

2.5 Invasive cervical cancer

Invasive cervical cancer mainly metastasizes through local invasion into the parametrium, vagina, uterus, bladder and rectum, but can also spread via lymph nodes to distant locations, such as the lungs, liver and skeleton. Abnormal bleeding is the cardinal symptom of invasive cervical cancer; however, early-stage disease is often asymptomatic. Common symptoms in advanced-stage disease include vaginal discharge and pain due to tumors or metastasis causing compression of organs and nerves.

The first cervical cancer staging system was developed by the Federation of Gynecology and Obstetrics (FIGO) in 1958. Since then, the FIGO staging system has been revised multiple times and the most recent update from 2018 determines stage based on clinical, radiological and pathological findings.

Stage I requires that the carcinoma be restricted to the cervix, with either microinvasion (IA) or larger tumor mass, but still confined to the cervix (IB). Stage II occurs when the carcinoma extends beyond the cervix, but has not spread to involve the lower third of the vagina or the pelvic wall. Stage III is defined as carcinoma invasion extending to the lower third of the vagina and/or the pelvic wall and/or any of the following: hydronephrosis, pelvic invasion, and para-aortic lymph node metastasis. Stage IV occurs when disseminated disease is present – the carcinoma invades the urinary bladder or rectum (IVA), or distant organs (IVB) (62).

The Tumor, Node and Metastasis (TNM) system developed by the American Joint Committee on Cancer (AJCC) is another staging system. The most recent version of the TNM from 2018 is well aligned with the FIGO staging system (63).

First-line treatment for cervical cancer includes surgery, radiation therapy and chemotherapy, depending on stage. Surgical treatment procedures like conization and hysterectomy are usually appropriate for early-stage disease (62). Survival rates are stage-dependent; overall 5-year survival for stages I, II, III and IV are 76-95%, 63-70%, 37-40% and 14-24%, respectively (64).

2.6 Risk factors

2.6.1.1 Sexual behavior

Sexual behavior affects the risk of HPV exposure and acquisition, since HPV is an STI. The risk of high-grade cervical dysplasia and cervical cancer increases with number of sexual partners (65, 66). Compared with one partner, six or more sexual partners doubles the risk of cervical cancer. Early age at first coitus is also a risk factor for high-grade cervical

dysplasia and cervical cancer, since it may increase lifetime duration of HPV infection. Young age at first coitus might serve as a marker for increased risk of HPV exposure (66).

2.6.1.2 Herpes simplex type 2 (HSV-2)

Infection with herpes simplex type 2 (HSV-2) has inconsistently been reported as a co-factor for development of cervical cancer. Smith et al. found in 2002 that HSV-2 seropositivity was elevated among cervical cancer patients compared with controls. After adjustment for potential confounders, they found that HPV-positive women who were seropositive for HSV-2 were at more than twice the risk of developing cervical cancer. They concluded that co-infection with HSV-2 infection in addition to HPV may increase the risk of cervical cancer (67). In contrast, a 2011 study by Dahlström et al. found little or no association between HSV-2 infection and cervical cancer risk. However, they found that co-infection with chlamydia trachomatis (CT) nearly doubled the risk of cervical cancer (68). It has been reported that CT coinfection increases the risk of acquisition and persistence of HPV, thereby increasing the risk of cervical cancer. The CT bacterial infection weakens the immune system and disrupts the cervical epithelium, thereby facilitating HPV entry into the cells and setting the stage for development of cancer (69).

2.6.1.3 Oral contraceptives (OC)

The association between use of oral contraceptives (OCs) and cervical cancer has been questioned. Some reports have shown that use of OCs for more than five years increases the risk of cervical cancer when compared with women who have never used OCs (70). Nevertheless, with cessation of OC use the increased risk of cervical cancer declines over time (71). Use of OCs for more than fifteen years compared with never-use showed an increased risk of high-grade cervical dysplasia (Hazard ratio [HR] 1.6) and cervical cancer (HR 1.8) associated with duration of OC use, according to a large cohort study including more than 300 000 women with previously confirmed OC use. The mechanism behind this association is not fully understood, though one explanation may be that hormones, especially progesterone, may interact with hormone receptors in the cervix to stimulate degradation of p53 by enhancing HPV expression of oncogenes E6 and E7 (72).

2.6.1.4 Smoking

The first report that smoking increases the risk of cervical cancer was hypothesized by Winkelstein et al. in 1977 (73). Nearly 25 years later, Plummer et al. showed that smoking acts as a cofactor in HPV-positive women to cause precancerous lesions and cervical cancer, resulting in a greater than twofold risk of cervical cancer among smokers

compared with non-smokers (74). A 2023 meta-analysis that included 109 studies confirmed the association between smoking and increased risk of high-grade cervical dysplasia and cervical cancer. Current smokers compared with former smokers demonstrate a 2.11 (95% CI, 1.85–2.39) relative risk of developing high-grade cervical dysplasia and a 1.70 (95% confidence interval [CI], 1.53–1.88) relative risk of developing cervical cancer. The analysis also found an association between smoking and cervical cancer in patients without HPV, which increased significantly with smoking intensity and declined after smoking cessation (75). This finding is in line with a prospective European study of 300 000 women (76). Wen et al. included almost 280 000 women who had never smoked, but were subjected to passive smoking. They were followed for a median of eleven years, during which time nearly 1100 cervical cancer cases were reported. Passive smoking was associated with elevated risk of cervical cancer, increasing risk by up to 29% (77). The risk of dying from cervical cancer is 21% higher among smokers than non-smokers; unfortunately, few patients cut back or quit smoking during treatment (78). The mechanism underlying the association between smoking and cervical cancer is complex and several explanations are plausible. One is that smoking weakens the immune system. Another is that carcinogens found in smoke interact with HPV-infected cells, where oncoproteins block apoptosis (79).

2.6.1.5   Immunosuppression

Immunosuppression, including genetic disorders, iatrogenic immunosuppression and infection with human immunodeficiency virus (HIV), are known risk factors for persistent hrHPV infection (80). Women with HIV are at increased risk of HPV infection, high-grade cervical dysplasia and cervical cancer (81). The risk of dying from cervical cancer is twice as high in HIV-positive women than in HIV-negative women (82). A meta-analysis of 38 studies that evaluated the relationship between HPV acquisition and HIV showed that the relative risk of HPV acquisition among HIV-positive women is 2.64 (95% CI 2.04–3.42) compared with HIV-negative women and increases with high HIV viral load and low CD4 count. The incidence of cervical cancer among HIV-positive women was 4.1 (95% CI 2.3–6.6) times higher than in the general population (83). Immunodeficiency caused by HIV has a negative impact on HPV acquisition and clearance rate (18). The risk of persistent hrHPV is increased among women living with HIV (84). The most frequent hrHPV types among HPV-positive women are HPV16, HPV52 and HPV35. Multiple hrHPV infections and hrHPV not targeted by the vaccines are common among women living with HIV (85).

2.6.1.6 Age

Approximately 30% of cervical cancers occur in women over age 60. This group has a poor prognosis, with a mortality rate of 70%, since cervical cancer often goes undiagnosed until reaching an advanced stage. With increasing age, the TZ migrates higher up into the cervical canal, which complicates accurate cytology. Persistent HPV infection is found in a large proportion of elderly women, for whom cytology is not an appropriate test due to very low sensitivity (86, 87). Testing for HPV is more sensitive than cytology in detecting precancerous cervical lesions, especially when used among women aged 35 or older (88). Women with hrHPV-positive tests demonstrate higher prevalence of histologically confirmed CIN2+ (86, 87). Hence, women who are HPV-positive by the end of the screening program are included in an extended screening program (30).

Difficulty in clearing HPV-infection increases with age. There is a bimodal distribution in the incidence of peak cervical dysplasia, with one peak occurring among women aged 20-25 years and the second among women aged 45-50 (89). HPV prevalence follows the same bimodal pattern. Since HPV infection is usually transient, the specificity of HPV screening is higher among women aged 35 or older than in younger women. Efficacy of HPV testing should be maximal when performed on women in their 30s since the peak of age-specific incidence of cervical cancer occurs among women in their 40s (90). Women older than 35 years are at significantly higher risk for persistent HPV infection post-conization, suggesting older age as a predictive factor for recurrent disease (91, 92).

Knowledge concerning HPV prevalence is limited among women aged 60 or older. However, the data indicate that HPV prevalence within this patient group is 5.5% (93), which is supported by other studies (86, 94, 95). True CIN2+ prevalence among older women is unknown. Women older than 60 have higher frequency of unclear margins and are at higher risk of recurrent disease. A more aggressive treatment strategy for this patient group is suggested, in which preservation of reproductive potential does not have to be considered (96, 97).

The risk of cervical cancer in women with history of high-grade cervical dysplasia is twofold compared with the general population and this risk remains elevated for more than two decades (56, 98). Additionally, for women over age 60, this risk is even higher, suggesting the need for lifelong surveillance (98). In countries with cervical screening programs, the reduction of cervical cancer is mainly seen among women aged 30-60 years. After termination of such screening, women aged 60 or older often request continued cervical cancer screening. Once these women exit the screening program, they may be misled into thinking they are no longer at risk of developing cervical cancer (86).

2.7 Prevention of cervical cancer by screening

Cervical screening programs aim to prevent cervical cancer through early detection and treatment of precancerous lesions (99). Since the introduction of cytology-based cervical screening programs, incidence and mortality rates have dropped dramatically (100, 101).

2.7.1 Cytology-based screening

The first reports concerning the Papanicolaou smear (Pap smear) can be traced to 1928 (102). The Pap smear, also known as conventional cytology, has been in common use for cervical screening programs since the 1960s (103). However, conventional cytology is associated with false-negative and false-positive results due to inadequate manual sampling technique.

Liquid-based cytology (LBC) was introduced as an alternative and offers certain advantages over conventional cytology. An automated process is used in sample preparation and several studies have shown higher detection rates of CIN and lower rates of uninterpretable results (104).

Sensitivity for detection of CIN2+ is reportedly 47% for conventional cytology, compared with 66% for LBC (103). Furthermore, the residual liquid can be used for reflex testing with HPV DNA. This higher sensitivity and potential to investigate for biomarkers makes LBC the method of choice for use in cervical screening programs (30, 105).

2.7.2 HPV testing

Despite the above technologies, the decline in incidence of cervical cancer has stagnated over the past decade (106–108), underscoring the need for new methods and strategies in cervical cancer screening. Testing for hrHPV is positive in 99.7% of all cases of cervical squamous cell carcinomas and in 94–100% of cervical adenocarcinomas (10). Given the important role of HPV in malignant transformation, researchers have developed DNA- or RNA-based methodology as alternatives to traditional cytology-based screening for detection of precancerous and early cancerous lesions.

Primary screening for hrHPV has reduced the incidence of cervical cancer by 60–70% compared with cytology-based screening (9, 101). HPV testing has higher sensitivity than cytology, but lower specificity, which is why cytology is used in some countries to triage HPV-positive women for colposcopy (6).

HPV testing is a sensitive, objective, nonmorphological screening method for cervical dysplasia and cervical cancer (109). Longer screening intervals are appropriate in HPV

testing compared with cytology screening since progression from a positive hrHPV test to cancer takes longer than such progression from precancerous cells (110). There is extensive evidence that HPV-based primary screening provides better protection against cervical cancer than cytology-based screening (111–113).

In 2015, the European guidelines were updated to strongly recommend HPV testing over cytology for primary screening (112). In 2021, the World Health Organization (WHO) also updated their guidelines for cervical cancer screening and now recommend HPV testing as the primary screening method (114). For HPV-negative women, the European guidelines recommend screening intervals of at least five years, which can be extended for up to ten years, depending on medical history (115).

2.7.3 Self-sampling for HPV

Screening program non-attenders are at increased risk of developing cervical cancer and most cervical cancers actually occur within this group. Thus, encouragement of screening and follow-up is vital. In fact, in recent years screening attendance has plateaued or even declined in some high-income countries. Use of HPV testing may potentially expand screening adherence (116, 117).

A large 2018 meta-analysis of 56 accuracy studies and 25 patient trials showed that self-sampled HPV testing based on the polymerase chain reaction (PCR) has comparable sensitivity to samples collected by trained personnel for detection of CIN2+. Mailing out self-sampling kits is a more effective strategy than sending invitations to screening program non-attenders (116).

2.7.4 Swedish settings

In Sweden, all resident women aged 23–70 years are included in the screening program. Women aged 23–49 years are screened every five years and women aged 50–64 years every seven years. HPV analysis is the primary screening test and positive results are divided into three subcategories: low-oncogenic HPV types (33/39/51/56/59/66/69), intermediate-oncogenic HPV types (31/33/52/58) and high-oncogenic HPV types (16/18/45). For HPV-positive women over age 33 cytology is used as a reflex test, regardless of oncogenic HPV subcategory. In cases of women under age 33 who are HPV-positive for intermediate- or high-oncogenic HPV types, cytology is used as a reflex test, but if positive for only low-oncogenic HPV types they resume routine screening with no further investigation. HPV analysis as a primary screening tool for women over age 30 was introduced in 2017 and in 2022 it was decided that HPV screening should be the primary test for all women in the program. Invitations to cervical screening ends at 70 years of

age, except for those who are HPV-positive or have previously been treated for high-grade cervical dysplasia (30).

In Sweden, self-sampled HPV testing (ssHPV) was incorporated into the national screening program in 2022. An HPV-self sampling kit is sent to the home address of all women who fail to participate in the cervical screening program and who are at least four years past the prescribed screening interval. Women who are positive on the ssHPV test will be referred within four weeks to a clinician for cytology testing. Unforeseen factors, such as the COVID-19 pandemic, may have a negative impact on adherence to preventive screening programs such as cervical screening. In such contexts, HPV self-sampling could serve an especially important function by maintaining a high participation rate, thereby lowering risk of morbidity and mortality from cervical cancer. In a randomized trial in Stockholm, women who had not participated in the screening program for at least ten years were sent either annual reminders, an ssHPV kit, or the opportunity to order such a kit. The participation rate among women receiving an ssHPV kit was 18.7%, while participation among women receiving annual reminders was only 1.7% (30).

Colposcopy, a gynecological examination of precancerous lesions using a microscope-like tool, is performed on women with a positive reflex test. Colposcopy entails application of acetic acid and iodine solutions to the outer surface of the cervix, known as the portio. The portio is assessed according to the Swedescore grading system where five variables are scored 0-2 points (acetowhiteness, margins, vascular patterns, lesion size and iodine uptake). Scores of 1-5 indicates the presence of low-grade cervical dysplasia and scores of 6-10 indicates the presence of high-grade cervical dysplasia (30, 118). Punch biopsies are taken from suspected precancerous lesions and sent for histopathological analysis (30).

The colposcopic examination is subjective and accuracy will vary with the experience of the colposcopist, as well as with disease prevalence (119). A number of studies have addressed the accuracy of colposcopy for detection of high-grade cervical dysplasia. Mitchell et al. found the sensitivity and specificity for detection of cervical dysplasia to be 87-99% and 23-87%, respectively (120), whereas the corresponding figures for Mustafa et al. were 29-100% and 12-88%. Use of the Swedescore system mentioned above demonstrated 95% specificity for detection of HSIL or cervical cancer when the score is eight points or above (121).

2.8 Prevention of cervical cancer by vaccination

The potential to prevent cervical cancer has significantly improved since the introduction of HPV vaccines. In regions where HPV vaccination coverage is high, the prevalence and incidence of HPV, cervical lesions and condyloma have declined. Such declines were even

seen in unvaccinated people, suggesting protection through herd immunity (122). The three-dose vaccine regimen is nearly 100% efficacious when administered to HPV-negative women under the age of 25 years. More than 270 million doses in total have been administered and no major adverse effects have been observed (123).

Three licensed prophylactic vaccines are available: Gardasil, Cervarix and Gardasil-9. They are made from noninfectious virus-like-particles (VLPs), using the HPV L1 capsid proteins. VLPs contain no viral DNA genome and cannot cause infection or carcinogenesis.
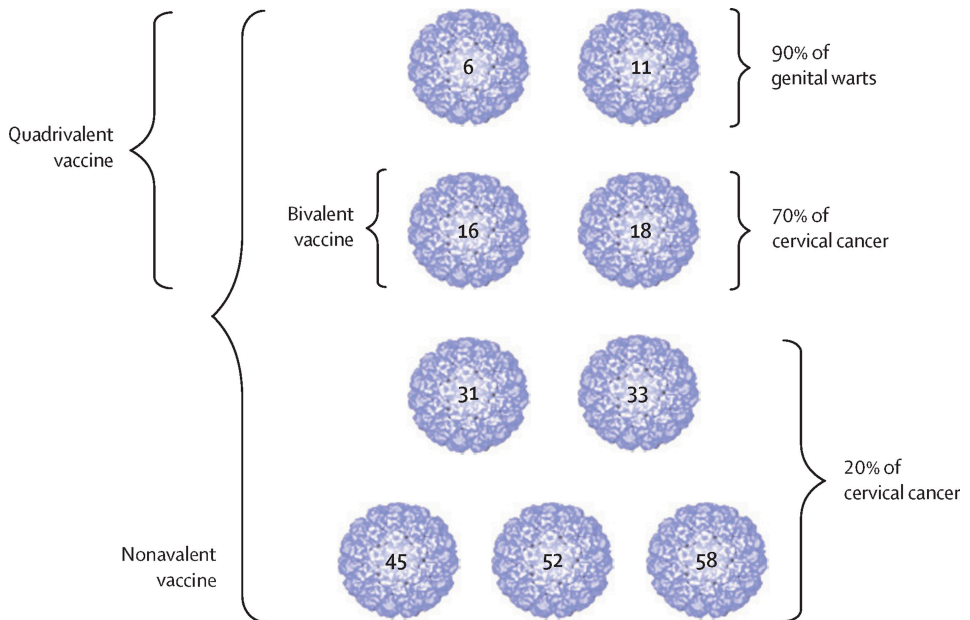
Figure 8. HPV vaccine types. Reproduced with kind permission from Schiller et al. (license number 5530690497867) (124)

Gardasil™ (Merck, West Point, PA, USA), approved in 2006, was the first HPV vaccine and is quadrivalent, with VLPs containing lrHPV (6 and 11) and hrHPV (16 and 18). Cervarix™ (GlaxoSmithKline, Rixensart, Belgium), approved in 2009, was the second HPV vaccine and is bivalent with VLPs containing hrHPV (16 and 18). Gardasil-9™ (Merck, West Point, PA, USA), a nonavalent vaccine with VLPs containing lrHPV (6 and 11) and hrHPV (16, 18, 31, 33,45, 52 and 58), is the most recent entry and received approval in 2014. Considering all invasive cervical cancers worldwide, HPV 16 and 18 account for approximately 70%, while HPV 31, 33, 45, 52 and 58 cause more than 15%. HPV6 and 11 cause more than 90% of all condylomas (26). Long-term assessment, ten years after administration of the initial dose of vaccine, shows that women aged 15-55 are still strongly seropositive for antibodies against HPV16, while seropositivity rates for antibodies against HPV18 decline over time (125). Nevertheless, mathematical modeling to assess long-term immunogenicity has been performed and predicted that antibodies against both HPV16 and HPV18 could be

detected more than 30 years post-vaccination and titers would be higher compared with natural infection (126). Furthermore, previous studies have shown cross-protection against HPV types not included in the vaccines. For example, it has been reported that bivalent vaccines cause some cross-protection against HPV 31 and 45. The underlying mechanism behind cross-protection is that L1 genes of the HPV types included in the vaccines share similarities with the HPV types that are not included (127).

In 2016, the Advisory Committee on Immunization Practice (ACIP) changed the recommendation from a three-dose regimen to a two-dose schedule for girls under age 15 (128). HPV vaccinations stimulate the humoral immune system to produce specific neutralizing antibodies against HPV antigens. Compared with natural infection, HPV vaccination produces up to 100 times higher antibody titers (129). Type of vaccination, age and gender determine the levels of antibodies produced, with higher titers seen among younger age groups (26). In December 2022, the WHO updated their recommendations on HPV vaccine. For females aged 9-20 living in poorer countries, off-label administration of a single dose of vaccine in an otherwise two-dose schedule provides efficacy and durability of protection against cervical cancer. Between 2019 and 2021, first-dose HPV coverage fell by 25% to 15% and a single-dose schedule is expected to improve vaccination access (130). A 2021 study from India reports that a one-dose schedule of HPV vaccine against persistent HPV16 and HPV18 provided 95.4% efficacy (95% CI = 85.0% to 99.9%) (131).

The beneficial effects of HPV vaccination have become clear, especially among females in regions of high vaccine coverage who were vaccinated prior to HPV exposure. Studies have shown a reduction of up to 85% for HSIL, 45% for LSIL and 90% for condylomas. The estimated efficacy of a single-dose regimen (or more) of HPV vaccine was over 80% (132, 133). A 2020 Danish study showed comparable efficacy for quadrivalent HPV vaccine against HSIL or worse pathology among women vaccinated with one, two, or three doses before age 16 (134). However, as mentioned above, efficacy may vary by age. Vaccination is more efficacious if administered before infection occurs, for which reason the recommended age for HPV vaccination is 11-12 years. HPV vaccination is approved for use in adults up to age 45, but since the advantages decline after age 26, vaccination is not routinely recommended for people over age 26 (135). The incidence of cervical cancer in vaccinated vs. unvaccinated individuals was 6.7 per 100 000 and 11.3 per 100 000, respectively. The incidence of cervical cancer remains low even with increasing age (at 0.01%) if vaccine is administered before age 16. However, among unvaccinated women and women who were vaccinated between ages 23 and 30, the incidence of cervical cancer increased rapidly at age 23 and peaked at age 30 (at 0.13%) (136).

Among males, HPV 16 and HPV18 causes 92% of all anal cancers, 89% of all oropharyngeal cancers and 63% of all penile cancers (137). The fist HPV vaccine for males was approved in 2014 by the European Medicines Agency (EMA). Approval was partly based on the

report that HPV vaccine protects against high-grade anal intraepithelial neoplasia (or worse) in men who have sex with men (MSM) (138). Furthermore, a significant reduction in anogenital condylomas was reported among males under the age of 20, indicative of herd immunity (139).

At least 107 countries worldwide have introduced a national HPV vaccination program, where boys are included in approximately 30% of such programs. Worldwide, about 15% of women and 4% of men are fully vaccinated against HPV (140). In Sweden, HPV vaccination (Gardasil) has been available to girls aged 10–12 since 2010 and to boys since 2020. Since 2012, a "catch-up" program providing free HPV vaccination to girls in the 18–26 age group has been available (141).

To accelerate eradication of cervical cancer, the FASTER strategy, launched in 2016, offers broad-spectrum HPV vaccine to females aged 25–45, with concomitant HPV screening for females over age 30. When following the FASTER strategy, screening frequency can be reduced for women who are HPV-negative at time of vaccination, since risk for development of cervical cancer is low in that group. This approach greatly reduces the need for screening. This strategy was based on the efficacy of HPV vaccination against HPV infection and related disease (range 85-100%) among adult HPV-negative women (142).

A modified Swedish concept, EVEN FASTER, was launched in 2021. This approach is based on use of the HPV reproductive rate to help identify appropriate age groups for targeting by the FASTER strategy (HPV vaccination and concomitant HPV screening) and as HPV is eliminated from circulation in Sweden, to screen the population at large for HPV-related diseases and precursor conditions that were previously induced by these viruses. The first step is to investigate and find the optimal maximum age for the FASTER strategy by analyzing the contribution of each age group to the circulation of HPV infection in the population. This can be done using the Basic Reproductive Number (R0). Sexual contact rates are age-dependent and in Sweden, R0 for women above age 30 is low, suggesting that if women under age 30 are unable to transmit the HPV infection, it would quickly disappear from the population. The HPV vaccination program was introduced in 2012 and participation rates have been high among birth cohorts aged 11-22 (80%). Therefore, currently the 23-30 year age group is mainly responsible for maintaining circulation of HPV infection in Sweden (143).

2.9 Treatment of cervical dysplasia

Although cervical intraepithelial neoplasia is usually asymptomatic, treatment is recommended for HSIL. However, low-grade dysplasia (LSIL) usually resolves spontaneously, for which reason treatment is not obligatory (144). Treatment of cervical

precancerous lesions is generally safe and effective. Ablative methods include cryotherapy and laser ablation, while excisional methods include loop electrosurgical excision procedure (LEEP), laser conization and cold knife conization (145). Cryotherapy can effectively be used to treat LSIL, with cure rates of 94% (146).

The recommended treatment for precancerous cervical lesions is conization, which removes abnormal cancerous tissue from the cervix in a cone-shaped piece (147, 148). A Cochrane review of 29 studies compared seven surgical techniques and found no significant differences in post-treatment outcomes in relation to persistent dysplasia. The overall treatment success rate was approximately 90%. Cryotherapy was mainly recommended for LSIL. LEEP provided the most reliable samples for histopathology and was also associated with the lowest morbidity (149).

Because of less perioperative bleeding, low risk of cervical insufficiency and relatively low cost, LEEP is becoming increasingly popular as a treatment of first choice (149). The cure rate when treating HSIL with LEEP is more than 90%, with a complication rate of approximately 10%. Cervical insufficiency is related to cone depth and the risk increases with increasing depth (150). Observational studies have reported a twofold increase in risk of premature birth associated with LEEP. According to a Swedish study, risk of premature birth increases regardless of cone depth; the risk increases by 15% for each additional mm over 10 mm. Repeated conization increases the risk of premature birth by up to four times (151). However, conization has no negative impact on fertility (152).

Laser conization is more expensive, causes more perioperative pain and is more time consuming than LEEP. Laser surgery also causes more thermal artifacts than LEEP. Except in cases where the CIN lesion is situated deeply and narrowly in the endocervical canal, LEEP is the surgical treatment of choice over laser conization (149). Another potential side effect of conization, regardless of surgical method, is cervical stenosis (150).

The newest treatment guidelines from the United States were updated in 2019 and recommend personalized risk-based management. A combination of test results and patient history are used to determine the risk of CIN3+, which serves as the basis for recommendations concerning surgical treatment, colposcopy, or surveillance. These guidelines were formulated to maximize cervical cancer prevention while minimizing the risk of overtreatment. The treatment algorithm begins by assessing whether the risk of CIN3+ is greater than 4%. If so, immediate treatment without colposcopy is recommended when the risk of CIN3+ is >60% and still acceptable when the risk of CIN3+ is >25%. Colposcopy is recommended if the risk of CIN3+ is 4-24%. In cases where the risk of CIN3+ is <4%, the 5-year CIN3+ risk should be estimated to determine surveillance interval. Should the 5-year CIN3+ risk fall below 0.15%, patients may return to routine screening at five-year intervals. Patients at a 0.15-0.54% risk of CIN3+ should be retested in 3 years and if risk at that time is >0.55%, testing should be repeated in 1 year. Moreover,

immediate treatment, without colposcopy, is warranted for nonpregnant women over age 25 in cases of a positive HPV16 test and HSIL on cytology. Observation is preferred for LSIL; however, treatment is acceptable if LSIL lesions persist for more than two years. After treatment of HSIL or AIS, continued HPV testing at three-year intervals is recommended for at least 25 years (153).

2.9.1   Swedish treatment guidelines

2.9.1.1   Persistent hrHPV infection

The Swedish guidelines recommend colposcopy, biopsy, cytology and HPV testing within three months of findings. Treatment is recommended if colposcopy and biopsy show HSIL. If instead LSIL is found, follow-up intervals are determined by the type of hrHPV infection. If results show persistent hrHPV and normal colposcopy and biopsy, women resume the routine screening interval as dictated by hrHPV findings. In cases of negative hrHPV with normal colposcopy and biopsy, women re-enter the routine screening program (30).

2.9.1.2   Positive hrHPV with cytology findings of LSIL/ASCUS

Colposcopy, biopsy, cytology and HPV testing should be done within three months of findings. When hrHPV test results are positive and colposcopy and biopsy show HSIL, recommendations are based on hrHPV type, age and findings of either CIN2 or CIN3. Conization treatment is recommended for all women >25 years with CIN3. Treatment is recommended for all women >25 years with CIN2 who test positive for HPV16/18/45. Treatment is recommended for all women ≥30 years with CIN2 and HPV 31/33/52/58 and all women ≥33 years with CIN2 and HPV 35/39/51/56/66/68. Women aged 23-29 years with CIN2 and HPV 31/33/52/58 should have repeat colposcopy with biopsy, cytology and HPV testing after six months. If CIN3 is then found, treatment is recommended, but if instead LSIL is found, testing should be repeated in one year. If CIN2 is found, testing should be repeated every six months for a maximum of two years, after which time treatment is recommended for persistent CIN2 (30).

For women ≤25 years with CIN3, treatment may be recommended after discussion and consensus by a multi-disciplinary team (MDT). Women ≤25 years with CIN2 who are positive for HPV 16/18/45 should have repeat testing within six months. Should these findings show CIN3, the MDT may recommend treatment. Should the findings instead show CIN2, repeat testing is recommended in six months, while treatment may also be recommended for findings of persistent HSIL. Repeated testing within one year is recommended for findings of LSIL (30).

2.9.1.3   Positive hrHPV with cytology findings of HSIL/ASC-H

The algorithm here is essentially the same as the one above pertaining to women with positive hrHPV and LSIL/ASCUS. The main difference is that treatment may be recommended without prior biopsy for women with a colposcopic Swedescore of >8 points, while excluding women ≤25 years and women <30 years with hrHPV 31/33/52/58. Another difference is that this algorithm recommends repeated testing within one year for women with normal colposcopy and biopsy. When findings are HPV-negative with normal cytology or LSIL/ASCUS, women resume routine screening, but if findings are HPV-positive or show HSIL, repeated testing is recommended with colposcopy, biopsy, cytology and HPV testing within three months (30).

For women ≤25 years with CIN3 on biopsy, conization treatment may be recommended after discussion and consensus by a multi-disciplinary team (MDT). Women ≤25 years with CIN2 and HPV 16/18/45 should undergo repeat testing in six months. If findings show CIN3, the MDT may recommend treatment. If findings show CIN2, repeated testing in six months is recommended, while treatment may also be recommended for findings of persistent HSIL. Repeated testing is recommended for findings of LSIL (30).

Conization should be carefully controlled so as to radically excise the lesion in one cone piece. Depending on the type of TZ (determined through colposcopic examination), different cone depths are recommended. The minimum cone length for effectively removing dysplasia in cervical crypts is 6 mm. Lack of clearly negative cone margins, especially toward the endocervix, is a predictor of treatment failure (30).

2.10 Recurrent cervical dysplasia post-conization

To date, there is no international consensus regarding optimal post-treatment follow-up and as to when women can safely resume routine screening. More intense follow-up post-conization is necessary (154). A well-established risk factor for treatment failure is unclear margins in the cone. Other risk factors that predict treatment failure and residual/recurrent disease post-conization include higher age, smoking, size and severity of the dysplastic lesion, as well as hrHPV type and persistence post-conization (96).

Residual disease is defined as CIN2+ diagnosed within two years post-treatment, and recurrent disease as CIN2+ more than two years post-treatment. Residual and recurrent disease are often grouped together when evaluating treatment success rate (155). Women with prior high-grade CIN are at elevated risk for recurrent/residual dysplasia and cervical cancer for a period of up to 25 years (98, 155). Several factors are thought to predict risk for treatment failure and recurrent/residual disease, including HPV status, cone size, positive resection margins, treatment modality and age (96). However, what factor most accurately identifies women at risk remains unknown.

Disease involvement of the resected tissue margins of the cone have been reported to increase the risk of recurrent CIN2+, but findings of high-risk HPV post-conization serve as a more accurate marker to identify treatment failure (97); recurrence of CIN2+ tends to appear earlier within this group compared with HPV-negative cases. Furthermore, risk of recurrent high-grade dysplasia is also elevated among women with positive margins compared with women with clear margins, regardless of age and HPV status. The histological area of disease involvement in the excised margin (endo/ectocervical and uncertain) can also predict treatment failure. Additionally, HPV is identified more frequently in involved margins compared with clear margins (96).

A 2022 Danish study included 11 684 patients who were treated with conization for CIN3 and then followed for up to 14 years. The risk of recurrent CIN2+ among HPV-positive women eight years post-conization was 12.5% (95% CI: 11.2–13.9) and for HPV-negative women 1.8% (95%CI: 1.5–2.1). Among women with positive and negative margins in the HPV-negative group, the risk of recurrent CIN2+ was 2.7% (95%CI: 2.1–3.5) and 1.3% (95%CI: 1.0–1.7), respectively. HPV-positive women showed the same pattern regarding positive and negative margins. These researchers concluded that HPV testing along with assessment of margin status had higher sensitivity but lower specificity than HPV testing alone (154).

Women with high-risk HPV and positive margins are at greater risk for recurrent disease than women with either unclear margins or HPV-positive status, as was recently shown in a meta-analysis that included 97 studies and a total of 44 446 women treated for CIN2+. Positive resection margins were associated with a relative risk of 4.8 for residual/recurrent CIN2+. However, the pooled sensitivity of HPV testing was much higher (91%) compared with margin status (56%), indicating that positive HPV status following treatment is a more accurate predictor of treatment failure than margin status. Given these new findings, information concerning HPV status is essential for accurate risk assessment and follow-up screening is recommended. Women with CIN2+ warrant special attention. The Swedish Society for Obstetrics and Gynecology recommends primary screening with HPV DNA testing while also continuing to recommend cytology co-testing (97).

3 Research aim

3.1 General aim

The overall aim of this thesis is to promote prevention of cervical cancer among women previously treated for precancerous cervical lesions by identifying risk factors for treatment failure and optimizing post-treatment surveillance and follow-up.

3.2 Specific aims

3.2.1 Study 1

To examine the long-term risk of residual and/or recurrent CIN2+ among women previously treated for CIN2+ and how this varies with margin status, comorbidity, post-treatment hrHPV and other factors.

3.2.2 Study 2

To identify risk factors that independently contribute to recurrent disease among women with a history of one initial treatment for CIN2+ by giving more extensive consideration to post-conization HPV data from a cohort of patients who received post-treatment follow-up.

3.2.3 Study 3

To assess the risk factors associated with recurrent/persistent AIS, as well as progression to invasive cervical cancer among women initially treated with conization for histologically confirmed AIS. The secondary aim was to evaluate adequacy of follow-up in order to suggest practical improvements to better protect this high-risk cohort.

4 Materials and methods

4.1 Study 1

This long-term prospective cohort study includes 991 women with histopathologically confirmed CIN2+ who underwent conization at Karolinska University Hospital (KUH) between 2000 and 2007.

Women diagnosed and treated with conization for CIN2+ at KUH between 2000 and 2007 were identified through their medical records using International Classifications of Diseases (ICD) diagnosis codes (N87.1; CIN2 or D06.9; CIN3) and Nordic Medico-Statistical Committee (NOMESCO) Classification of Surgical Procedure codes (LDC03; conization with diathermy or laser, or LDC00; conization with knife). Inclusion criteria were diagnosis of histopathologically confirmed CIN2/3 in patients prior to undergoing conization, with information on margin status of the excised cone.

Clinical data were obtained from medical records covering the period November 1, 2015 to November 30, 2017. These included age at surgery, any prior surgical treatment of the cervix, comorbidity, treatment modality, hrHPV status (when available), and retreatment, if any (local or hysterectomy). Comorbidity included conditions likely to interact with acquisition of hrHPV or CIN progression (autoimmune disorders, HIV and/or hepatitis B or C infection, malignancy, diabetes, genetic disorders, organ transplantation).

Pathology reports were used to gather a variety of information, including diagnosis prior to treatment (histopathology or cytology), cone biopsy findings, histopathology uncovered during follow-up or from local reconization biopsy samples and hysterectomy (if any), and primary exposure of interest as pertaining to margin status of cone biopsy (positive or negative; histological tissue involvement of positive margins – whether ectocervix, endocervix, both, or unknown). When both AIS and CIN were present, the lesion was coded AIS; when both ADC and SCC were present, it was coded ADC. Negative margins were defined as absence of high-grade dysplasia in surgical margins and material from endocervical curettage, according to standard practice post-conization in Sweden.

Information concerning primary outcome, histopathologically confirmed residual/recurrent CIN2+ (CIN2, CIN3, AIS, or worse), was obtained from medical records and linkage to the Swedish National Cervical Screening Registry, using unique personal identification numbers. The registry includes data for all cervical cytology and histopathology in Sweden (100% information on the most severe diagnosis), but does not include complete data on residual/recurrent disease outside the cervix. The present analysis identified residual/recurrent disease when such data were found in the medical records. All re-excision data were used to determine whether, and if so when, recurrent

CIN2+ had been detected. The results of histopathology at hysterectomy were also considered with respect to recurrent CIN2+.

At the time of the study, treatment modalities for CIN2/3 used at KUH were electrosurgery with contoured-loop excision of the transformation zone (C-LETZ), similar to LEEP, or, less commonly, electrosurgery with a diathermy needle; laser (CO_2) conization and rarely, knife excision. Most follow-up hrHPV testing was done at least 6 months post-conization.

4.2 Study 2

4.2.1.1 Study design, population and data collection

All patients who had received primary treatment with conization between October 2014 and January 2017 for histologically confirmed CIN2+ or AIS were eligible for participation in this study. Patients had been treated at the following hospitals: KUH, Danderyd Hospital and South General Hospital, all within Stockholm County, Sweden.

The study included a total of 532 patients. First follow-up visit, at six months post-treatment, took place at KUH. All 532 patients presented for the first follow-up. Each woman received information about the study procedures, including 1) self-collection of vaginal and urine samples for HPV testing, as previously reported (156), 2) completion of a questionnaire, as previously reported (157, 158) and 3) gynecological examination with colposcopy and cervical sampling for clinical follow-up. One patient declined participation. Two patients, while enrolled in the study, were found to have microinvasive SCC upon histopathological re-examination and were therefore excluded from further follow-up analyses. This brought the total number of patients in the present study down to 529.

4.2.1.2 First follow-up visit

The first follow-up visit included gynecological examination, colposcopy and cervical sampling by the clinician. One of the two gynecologists (Dr. Andersson or Dr. Mints), performed cervical sampling and colposcopy-directed punch biopsies when visible lesions were found. The biopsies were histologically graded with analyses done at KUH using CIN classification (44, 159). Patients with recurrent disease were referred for follow-up treatment, either re-excision or total hysterectomy, based on clinical evaluation and similar considerations. Participants were followed according to national guidelines using cytology co-testing.

4.2.1.3   Subsequent follow-up

Results from LBC and Cobas HPV from the first follow-up were used to determine subsequent follow–up. Patients were referred for a second follow–up when cytological abnormalities were discovered and/or the Cobas HPV result was positive. The second follow-up was usually scheduled one year after the first and was guided by the same standardized protocol (based on Swedish national guidelines). Under the Swedish national guidelines, patients could resume routine triennial screening if cytology sampling found negative intraepithelial lesions or malignancy (NILM) and the HPV Cobas findings were also negative. This routine screening was intended to include Cobas HPV testing, using a sample obtained by a clinician, and cytological examination, along with colposcopy performed at the discretion of the clinician.

4.2.1.4   Review of medical records

Clinical data that were obtained from medical records and carefully reviewed through December 2020, including age at surgery, comorbidity (conditions assumed to interact with HPV acquisition or CIN progression such as autoimmune disorders, malignancy, HIV or hepatitis infection, diabetes mellitus, genetic disorders, or organ transplantation), conization method, grade of dysplasia in the excised cone, number of resected cone specimens and margin status in the cone biopsy. Excisions were considered incomplete when dysplasia was found in the specimen margin, or termed "unclear" when margin status was uncertain. Assessment of unclear resection margins was further subdivided into: i) ectocervical only, ii) endocervical only, or iii) both margins unclear or uncertain. Diagnosed recurrent/residual disease was defined as histologically confirmed high-grade CIN on biopsy obtained through colposcopy at any of the follow-up examinations.

4.3 Study 3

4.3.1.1   Study design, population and data collection

Patients who had undergone primary conization between January 2001 and January 2017 at any of the three relevant Stockholm Hospitals (KUH, Danderyd, or South General) and with findings of histopathologically confirmed AIS in the excised cone were eligible for inclusion in the study.

The patients to be included in the study were identified from the Swedish National Cervical Screening Registry. Twenty-seven patients who were included in study 2 also participated in the present study. Thus, altogether we identified 84 patients who had undergone primary conization at one of the above-named Stockholm hospitals and in whom the results showed histopathologically confirmed AIS in the excised cone.

4.3.1.2 Review of medical records

The complete medical records for each patient were thoroughly reviewed through April 2022. We obtained information concerning age at surgery, treatment modality, smoking status (if reported, categorized as: current smoker, ex–smoker, or never smoker), comorbidity (conditions likely to interact with HPV acquisition or CIN progression such as autoimmune disorders, malignancy, HIV or hepatitis infection, diabetes mellitus, genetic disorders, or organ transplantation), grade of dysplasia in the excised cone and margin status in the cone biopsy. Clear margins were defined as having no high-grade dysplasia in surgical margins or in material curetted from the endocervix, according to standard practice post-conization in Sweden.

4.3.1.3 Review of post-conization follow-up data

The number of months between conization and the first gynecological follow-up, as well as the total number of post–conization follow-up visits were reported. The total number of years of reported gynecological follow–up visits and the total number of years that had passed without reported gynecological follow-up visits was documented.

The information obtained from follow-up cytology was categorized as either all normal, or at least one abnormal result. Abnormal results were then sub–classified as either exclusively low-grade versus high-grade (AIS or HSIL). Abnormal results were also classified as only glandular, only squamous, both glandular and squamous, or undefined atypical cells. All post-conization HPV results were documented and summarized as follows: all negative HPV results, at least one positive HPV result, at least one positive HPV16 or HPV18 result, and two or more positive HPV results.

All gynecological outcomes were documented. It was noted whether any reconization had been performed, whether hysterectomies were done and why, as well as the most serious histopathological post-conization finding. Disease recurrence was defined as patients with histopathologically confirmed AIS, HSIL, microinvasive carcinoma, or invasive disease. In contrast, patients with high-grade findings on cytology alone (AIS or HSIL), without histopathological findings, were classified as having likely recurrence.

4.3.1.4 Follow-up guidelines and procedures during the study

Early during the study, Swedish National Cervical Cancer Guidelines for follow-up of patients treated for high-grade CIN were based on margin status. Patients with negative margins underwent cytology after 6, 12 and 24 months and thereafter biennially. Any grade of dysplasia motivated referral for colposcopy. Women with unclear or uncertain margins were to be followed up with colposcopy-directed biopsy and cytology within 4-6 months,

or referred for reconization. More recently, HPV testing was included in the National Cervical Cancer Guidelines. Patients with demonstrated NILM on cytology and negative HPV at follow-up could resume routine triennial screening, as per the national guidelines.

4.4 HPV testing and cytology

4.4.1.1 Studies 1 and 3

At the time of the study, the hrHPV tests used at KUH were the Hybrid Capture 2 HPV DNA Test (QIAGEN, Gaithersburg, MD), Cobas 4800 (Roche Molecular Diagnostics, Pleasanton, CA), and Linear Array HPV Genotyping Test (Roche Molecular Systems, Alameda, CA). Results were considered to be positive if any potentially high–risk HPV (26, 53, 66, 68, 73, 82) types or known high–risk HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) types were identified.

Papanicolaou (Pap) smears were routinely in use during the early years of the studies, but in 2010 liquid-based cytology (ThinPrep®, Hologic, Marlborough, MA, USA) replaced earlier cytological methodology in Sweden.

4.4.1.2 Study 2

Study 2 used liquid-based cytology (ThinPrep®, Hologic, Marlbororgh, MA, USA) for cytopathological analysis and the Cobas 4800 assay (Roche Molecular Diagnostics, Pleasanton, CA, USA) for standard HPV testing. Samples from the endocervix were obtained with cervical brushes and from the ectocervix using plastic spatulas. The samples were transferred into PreservCyt liquid-based cytology (LBC) vials according to European guidelines. The Cytology Department at KUH performed LBC according to the Bethesda system. HPV DNA testing was performed on-site using the hospital's standard Cobas 4800 HPV (Roche Diagnostics) assay.

In addition, for purposes of participation in a different study unrelated to clinical decision-making, clinician-collected cervical samples (Abbott GmbH & Co.KG, Westbaden Germany), self-collected vaginal samples (VSS) and urine samples were analyzed for comparative HPV testing at first follow-up. The procedure entailed use of a multiplex real-time polymerase chain reaction (PCR) test that detects HPV16 and HPV18, as well as other high-risk HPV types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68. The results of this comparative testing are detailed in a previous study (156). In this study, the presentation of results from the Abbott clinician-collected cervical samples and VSS mainly reflect patients in whom recurrent disease was detected.

4.5 Statistical analysis

4.5.1.1 Study 1

Initial comparisons between patients with negative vs. positive/uncertain margins were based on use of the Pearson χ^2 test with salient dichotomizations for 2–by–2 analysis. Follow–up time was calculated beginning from date of conization until date of residual/recurrent CIN2+, death, deregistration, or end of study (December 31, 2016), whichever occurred first. There were no missing data in any of our variables (except for post–conization hrHPV, as previously noted). Kaplan–Meier plots were used to display the proportion of women with margin–positive/uncertain vs margin–negative findings who remained free of residual/recurrent disease and log–rank tests were used to compare differences. Twenty–five women with SCC or ADC on cone biopsy were excluded. The Cox proportional hazards model was used to calculate HR and CI regarding associations between margin status, as it pertains to the various locations of positive/uncertain margins, and residual/recurrent CIN2+. The model included relevant covariates that might also be predictors of residual/recurrent CIN2+, specifically age at surgery, previous conization, lesion severity obtained from baseline conization, comorbidity, and treatment modality. Additional Cox regression analysis was performed, stratified by hrHPV status. We calculated sensitivity and specificity for hrHPV and/or margin status as to how these relate to prediction of recurrent/residual CIN2+. SPSS (IBM version 25.0; IBM Corp., Armonk, NY) and Statistica (13.4.014/TIBCO–2018) were used for statistical analysis.

4.5.1.2 Study 2

A power analysis performed prior to the study calculated that 500 patients were needed for statistical significance at an alpha level of P<0.05. Initially, extensive univariate and bivariate analyses were undertaken. The latter was performed using the Pearson χ^2 test or Fisher's exact test if any expected cell was less than five, with one degree of freedom. All comparisons were two-sided. Salient dichotomizations were thereby made, as described in the Results section. Statistica (13.5.0.17/TIBCO-2018) and SPSS (IBM-version-25.0; IBM, Armonk, NY, USA) were used for statistical analysis. Multiple logistic regression was used to compute odds ratios (OR) and 95% CI with the detected recurrence of high-grade CIN serving as the outcome measure.

4.5.1.3 Study 3

To begin, comprehensive univariate and bivariate analyses were carried out. The latter were performed using 2-sample 't' tests, Mann-Whitney (MW) tests, Pearson χ^2 or Fisher's exact test if any expected cell was less than five. Unless explicitly stated otherwise, all

comparisons were two-sided. The result of the MW test was cited whenever the continuous or semi-continuous variable deviated from a normal distribution (skewness and/or kurtosis ≥1.5). For significant bivariate associations in which the outcome measure is detected recurrence of histopathologically confirmed CIN2+, sensitivity and specificity were computed with a 95% CI, along with negative predictive values (NPV) and positive predictive values (PPV). To compute OR and 95% CI, multiple logistic regression (MLR) analysis was used, with detected recurrence of histopathologically confirmed high-grade CIN as the outcome measure. For this statistical analysis, the 14.0.0.15 2020 TIBCO version of the Statistica software was used.

4.6 Ethical considerations

The Swedish Ethical Review Authority has approved all studies in this doctoral thesis. According to our ethical approval, patients do not need to provide informed consent before being included in the studies, except as otherwise specified (study 2). Patients who blocked access to their medical records were excluded. Patient autonomy may come into question when they are unable to decline participation. However, it is nearly impossible to obtain informed consent from such a large cohort. Therefore, I must share the view of the Swedish Ethical Review Authority that the potential benefits to be expected from this study override the concerns above and exceed any potential negative consequences. The cohort was not drawn from any vulnerable group, nor were any minors (under the age of eighteen) included.

There is minimal risk of physical harm associated with my studies, since they are undertaken as a registry study with no participant being subjected to extra examinations, tests, or procedures. The research had no impact on outcome for any patient (sampling, diagnosis, treatment, or follow–up).

Data management is likely the most sensitive aspect of my project, where risk of harm is greatest. All data are pseudonymized, never shared via any network, and have been stored on password–protected disks. The key is also under secure storage.

There is no significant impact on patient privacy and no sensitive data are presented for any individual patient since our studies are based solely on aggregated data. Consequently, no included patients are at risk of having their identity revealed.

4.6.1.1 Study 1

The study was approved by Stockholm's Ethical Review Board, which determined that participant informed consent was not required (Ref. no.: 168/ 03,2004–679/3,2010/944–32,2013/763–32,2014/2255–31/5,2017–2007/32). Nevertheless, we excluded women who had opted to block access to their medical records.

4.6.1.2 Study 2

All participants were assured of complete confidentiality and full freedom to withdraw from the study at any time with no consequences whatsoever. The informed consent included permission to review the patient's medical records. Options for informed consent were: agree to participate and decline to participate. All participants provided written informed consent. Karolinska Ethics Committee approved the study protocol (approval ref. no.: 2006/1273-31, 2014/2034-3).

4.6.1.3 Study 3

The study was approved by the Regional Ethics Committee in Stockholm, based at Karolinska Institutet, who determined that informed consent from participants was not required (Ref. no.:168/03, 2004-679/3, 2010/944-32, 2013/763-32, 2014/2255-31/5, 2017-2007/32). Nevertheless, patients were given the option to block access to their medical records, in which case they were excluded from the study. In the present study, none of the women blocked access to their records. Thus, no patients were excluded for this reason.

5 Results

5.1 Study 1

5.1.1.1   Characteristics of the study sample

In total, 991 women were included and 84 excluded from the study. Reasons for exclusion were missing data pertaining to histopathologically confirmed high-grade cervical dysplasia (n=43), margin status (n=18) and conization (n=5). In addition, 18 women denied access to their medical records (n=18). At the conclusion of the study, approximately 4% of patients were deceased and 2% were deregistered. Median age of patients was 33 years (range 19–94 years). The three most common comorbidities in the cohort were autoimmune disease (3%), HIV (1.7%) and hepatitis B and/or C (1.2%). However, the majority of the cohort (91%) demonstrated no underlying comorbidity. Just over two-thirds of these women were treated by contoured loop excision of the transformation zone (C-LETZ) (or electrosurgery by diathermy needle) and the remaining third by laser conization. Information on hrHPV status post-conization was available for 22% of the women. Diagnoses from baseline conization were as follows: CIN3 (63.1%), CIN2 (21.6%), CIN1 (6.8%), no dysplasia (4.2%), SCC/ADC (2.5%), AIS (1.6%) and other (0.2%). During follow-up, 109 patients underwent reconization, of whom 48.6% were found to have CIN2+ on biopsy. Furthermore, 91 women underwent hysterectomy; residual/recurrent dysplasia was the most common reason.

5.1.1.2   Margin status on baseline conization

Women with a history of positive/uncertain margins underwent reconization significantly more often than women with negative margins. Negative margins were found in 65.2% of the cohort, while 4.2% had no dysplasia at all, leaving 30.6% of the cohort with positive/uncertain margins, mostly found in the endocervical region only (Table 1).

Table 1: Margin status on baseline cone biopsy		
	n	**%**
Margin status		
Negative	646	65.2
Positive/uncertain at any margin	303	30.6
• Endocervical	127	12.8
• Ectocervical	71	7.2
• Both	61	6.2
• Uncertain	44	4.4
No dysplasia on cone biopsy	42	4.2

5.1.1.3   Follow-up data according to margin status in relation to recurrent disease

Recurrent/residual high-grade cervical dysplasia was significantly more common in women with positive/uncertain margins than in women with negative margins. The former group had a higher probability for detection of recurrence within one-year post-conization (Table 2).

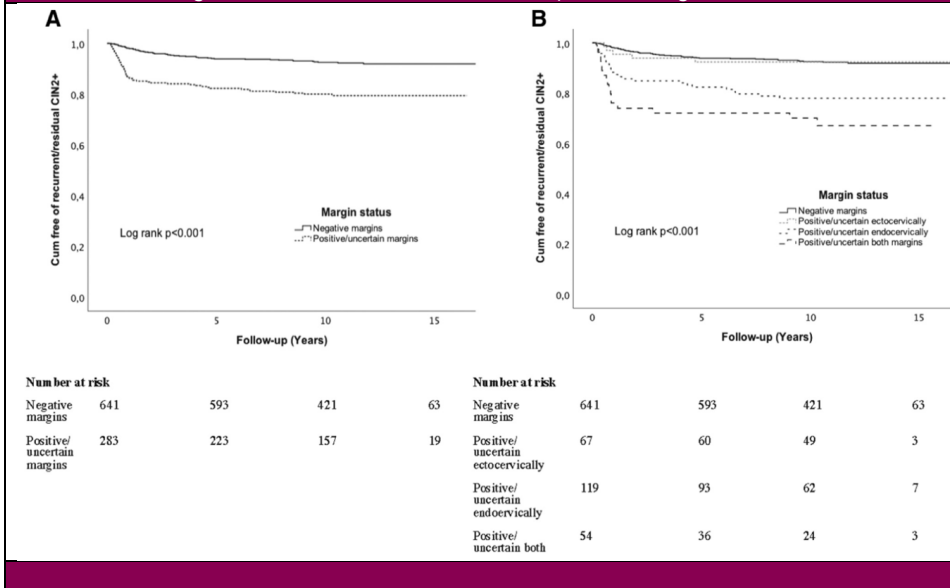| Table 2: Residual/recurrent high-grade (or worse) cervical dysplasia at follow-up						P value
	Total		**Margin status**			
	Number	**%**	**Negative (n)**	**Positive/ uncertain (n)**	**No dysplasia (n)**	
Residual/recurrent CIN2+						<0.001
No	855	88.5	592	226	37	
Yes	111	11.5	49	57	5	
Time lapse between surgery and CIN2+ (years)						<0.001
0–1	51	45.9	12	38	1	
1–3	26	23.4	18	7	1	
3–5	16	14.4	9	5	2	
5 or more	18	16.2	10	7	1	

The 25 women diagnosed with SCC/ADC in the original cone biopsy were excluded from these analyses. Unless otherwise specified, Pearson χ^2 analysis was used to compare pathology findings of negative margins with findings of positive or uncertain margins on conization.
CIN = cervical intraepithelial neoplasia. NS = statistically nonsignificant (P > .10).

Figure 1 shows follow-up findings based on margin status in relation to recurrent/residual CIN2+. Positive/uncertain margins, especially when both margins were positive/uncertain, led to significantly higher cumulative recurrent/residual CIN2+, compared with negative margins.

Figure 1: Recurrent/residual cervical intraepithelial neoplasia or worse (CIN2+) in relation to margin status.
A, Kaplan–Meier plot of cumulative percentage of patients free of residual/recurrent CIN2+ by margin status.
B, Kaplan–Meier plot of cumulative percentage of patients free of residual/recurrent CIN2+ by differences in margin status and anatomical location of positive margins. Cum=cumulative.

5.1.1.4 Cox regression model for residual/recurrent high-grade cervical dysplasia (or worse) according to margin status

Positive/uncertain margins were associated with residual/recurrent high-grade cervical dysplasia after adjusting for age at conization, prior conization, comorbidity, severity of lesion and type of surgery.

When endocervical margins were positive, risk of recurrent high-grade cervical dysplasia increased by almost 300% compared with women who had negative endocervical margins. Furthermore, when both endo- and ectocervical margins were positive, the risk of recurrence increased by almost 500%. Comorbidity was a significant predictor for recurrent/residual CIN2+ (table 3). No significant association was found between recurrence and age at conization, type of surgery and prior conization. Residual/recurrent CIN3+ was found in 63 women and results from an additional Cox regression in which CIN3+ served as the endpoint similarly showed significant findings as described above (except for comorbidity, which showed borderline significance [P = .006]).

Table 3: Cox regression model for residual/recurrent high–grade cervical dysplasia (or worse)

	Adjusted HR[a]	95% CI	P value
Margin status			<0.001
Negative	1		
Not applicable	1.94	0.51–7.43	
Positive/uncertain	2.67[b]	1.81–3.93[b]	
• Positive/uncertain: ectocervical	0.96	0.38–2.42	
• Positive/uncertain: uncertain	2.84[b]	1.39–5.81[b]	
• Positive/uncertain: endocervical	2.72[b]	1.67–4.41[b]	
• Positive/uncertain: both	4.98[b]	2.85–8.71[b]	
Comorbidity			0.002
No comorbidity	1		
Comorbidity	2.23[b]	1.36–3.66[b]	

The 25 women diagnosed with SCC/ADC at baseline conization were excluded from these analyses.
CI = confidence interval. HR = hazard ratio.
[a] Hazard ratios are adjusted for all variables in the analysis; [b] Statistically significant HR and CI;

5.1.1.5 Cox regression model for residual/recurrent high–grade cervical dysplasia (or worse) according to follow-up hrHPV

The risk of recurrent/residual high–grade cervical dysplasia (or worse) increased by more than 250% among women with positive hrHPV and positive/uncertain margins compared with women who were positive for hrHPV and had negative margins (Table 4).

Table 4: Cox regression model for residual/recurrent high–grade cervical dysplasia (or worse) according to follow-up hrHPV status

	hrHPV positive (n=84)		hrHPV negative (n=105)	
	Adjusted HR[a] (95% CI)	P value	Adjusted HR[a] (95% CI)	P value
Margin status		0.031		0.455
Negative	1		1	
No dysplasia	18.74[b] (1.57–220.40)[b]		0.90 (0.05–15.22)	
Positive/ uncertain	2.56b (1.17–5.62)[b]		1.18 (0.40–3.49)	

The 25 women diagnosed with SCC/ADC in the baseline conization were excluded from these analyses.
CI = confidence interval. HR = hazard ratio. hrHPV = high–risk human papilloma virus.
[a] Hazard ratios adjusted for age at surgery, diagnosis on cone biopsy, treatment modality, and previous conization;
[b] Statistically significant HR and CI.

When excluding all cases without dysplasia, the sensitivity and specificity of margin status in predicting recurrent/residual high–grade cervical dysplasia (or worse) were 53.8% and 72.4%, respectively. In all, recurrence was detected in 42% of the women with positive hrHPV and positive/uncertain margins, and in 29% of the women with positive hrHPV but negative margins. Sensitivity and specificity of positive hrHPV findings for predicting recurrent disease when margins are positive/uncertain were 66.7% and 64.4%, respectively. Sensitivity and specificity of positive hrHPV findings for predicting recurrent disease when margins are negative were 61.5% and 65.5%, respectively. And finally, sensitivity and specificity of positive hrHPV findings and/or positive/uncertain margin status for predicting recurrent disease were 66.7% and 64.4%, respectively.

5.2 Study 2

5.2.1.1 Characteristics of the study sample

Mean age at time of conization of the 529 patients included in the study was 34.3 years; 35.5% were <30 years, 50.1% were 30–44 years and 14.4% were ≥45 years. The majority (85%) were treated with C–LETZ, 14.6% were treated by laser conization and three patients with ablation. Margin status was as follows: both margins clear (73%), both margins unclear/uncertain (10.6%) and only one margin clear (16.4%). Just over two–thirds of the cohort had high–grade cervical dysplasia or worse in the histological results from the excised cone. One or more comorbid diagnoses were found in 25.7% of the patients.

5.2.1.2 Single characteristic data from first, second and routine follow-up.

First follow–up: More than 50% of the cohort presented for the first follow–up within six months post–conization, while 100% of the cohort had attended the first follow–up by 15 months. Complete data for both cytology and HPV results at first follow-up were available. NILM was found in 86% of the women, while HPV status was positive in 16.3%. In all, 7% had both abnormal cytology and positive HPV findings. Recurrent high–grade cervical dysplasia was detected in four women.

Second follow–up: In all, 108 of the 121 referred patients presented for the second follow-up. The four patients who had recurrent high–grade cervical dysplasia (or worse) detected at the first follow-up were excluded. Most of these patients presented for the second follow–up within one year of the first follow–up and over 90% had presented within two years. Cytology results showed NILM in 69%, abnormal findings in 29% and were missing for three women. HPV results were positive in 40%, negative in 47% and unavailable for 13% of the women. Recurrent high–grade cervical dysplasia was detected in nine women.

Routine follow–up: In all, the 404 women with NILM and negative HPV at first follow–up were referred for routine triennial screening (among whom 85.4% attended). Ninety–two women without detected recurrence at the second follow–up presented for subsequent routine screening. Thus, a total of 437 women continued with follow–up. At later screening events, cytology showed NILM in 345 women, abnormal findings in 34 and results were missing for 58. Thirty–six women were HPV–positive, 299 were HPV–negative and HPV results were missing for 102 women. Recurrent high–grade cervical dysplasia was detected in nine patients.
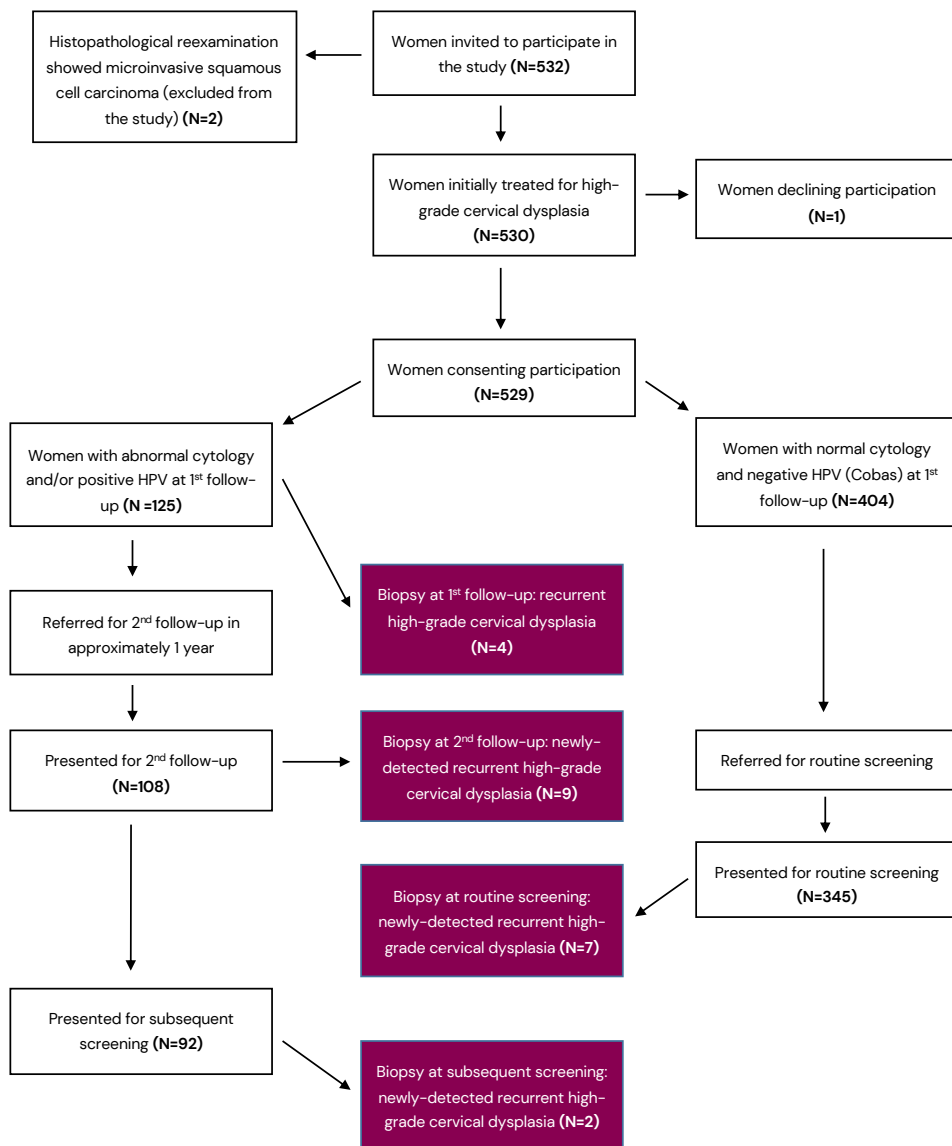
Figure 1. Flow chart for study participants. Blue boxes show women with recurrence detected at follow-up. HPV=high-risk human papillomavirus.

5.2.1.3   Characteristics of the study sample with detected recurrent disease

First follow-up: All four patients with recurrent disease detected at first follow-up showed HSIL on biopsy and all were HPV-positive.

Second follow-up: Two of the nine patients who had recurrent disease detected at second follow-up showed AIS on biopsy, while the remaining seven showed HSIL. One of

the two patients with AIS had been HPV–negative (Cobas) and the other had been HPV–positive (Cobas) at first follow–up, while both patients were HPV–positive at second follow–up. The seven patients with HSIL were HPV–positive at first follow–up and six of these seven were assessed at second follow–up, all of whom were HPV–positive.

Subsequent follow–up: Of the nine patients with recurrent high–grade cervical dysplasia detected in subsequent–/routine screening, two had AIS and seven had HSIL. Both patients with AIS were HPV–negative (Cobas) at first follow–up, but were found to be HPV–positive later during routine screening. Five of the seven patients with HSIL had NILM and were HPV–negative (Cobas) at first follow–up, but routine screening found three women who were HPV–positive, one who was still HPV–negative, while data were missing for the last patient. The other two patients with HSIL presented for second follow–up motivated by abnormal cytology as well as positive HPV at first follow–up.

5.2.1.4   Comparisons between women with and without detected recurrence (Table 1)

Detected recurrent high–grade cervical dysplasia was significantly more often found among women aged ≥45. No significant differences were observed among the groups regarding treatment modality, comorbidity, cone histology, or number of cone pieces. Nevertheless, unclear/uncertain margins were found in 64% of women with recurrent disease, compared with 25% of women without recurrence. Abnormal cytology at first follow–up, second follow–up (excluding women with detected recurrence at first follow–up) and subsequent follow–ups was significantly more common among women with recurrent disease than among those without recurrence (59% vs. 12%, 91% vs. 22% and 44.4% vs. 8.1%, respectively). Moreover, women with recurrent disease were significantly more often HPV–positive on Cobas at each follow–up compared with women without recurrence (64% vs. 14%, 100% vs 40% and 87.5% vs. <1%). Persistent HPV (Cobas) at first and second follow–up was found in all eight patients with detected recurrence, whereas approximately half of the women without recurrent disease who were HPV–positive (Cobas) at first follow–up had become HPV–negative (Cobas) at second follow–up.

Table 1. Comparisons between women with and without detected recurrent high–grade cervical dysplasia

Variable	No recurrence of CIN2+	Recurrent CIN2+	P value
Age at conization, years			<0.01
< 45	440	13	
≥ 45	67	9	
Margin excision status[a]			<0.001
Both clear	378	8	
Ectocervical unclear/uncertain	41	1	
Endocervical unclear/uncertain	38	7	
Both unclear/uncertain	50	6	
Cytology at 1st follow–up			<0.001
NILM	444	9	
Abnormal	63	13	
Cytology at 2nd (referred) follow–up[b,c]			<0.001
NILM	73	1	
Abnormal	21	10	
Cytology at routine follow–up[b,d]			<0.01
NILM	340	5	
Abnormal	30	4	
hrHPV at 1st follow–up (Cobas)			<0.001
Negative	435	8	
Positive	72	14	
hrHPV at 2nd (referred) follow–up (Cobas)[b,e]			<0.001
Negative	51	0	
Positive	34	9	
hrHPV at routine follow–up (Cobas)[b,f]			<0.001
Negative	298	1	
Positive	30	6	
Persistent hrHPV positivity (Cobas)[b,g]			<0.01
No (HPV positive at 1st follow-up, HPV negative at 2nd follow-up)	27	0	
Yes (HPV positive at 1st & 2nd follow-up)	28	8	

Data were analyzed using two-tailed Pearson's χ^2 or Fisher's exact test if any expected cell is <5, with one degree of freedom. [a]Margin status dichotomized to both margins clear vs. one or both margins unclear or uncertain. [b]Assessments subsequent to the 1st follow-up exclude the patients in whom recurrence was detected prior to that follow-up. [c]No cytology data available at 2nd follow-up for three patients without detected recurrence. [d]No cytology data available at routine follow-up for 58 patients without detected recurrence. [e]No HPV data available at 2nd follow-up for 12 patients without detected recurrence and for two patients with detected recurrence. [f]No HPV data available at routine follow-up for 100 patients without detected recurrence and for two patients with detected recurrence. [g]No HPV data available at 2nd follow-up for 12 patients without and 2 patients with detected recurrence, who tested positive for HPV at 1st follow-up. hrHPV = high-risk human papillomavirus. NILM = Negative for intraepithelial lesions or malignancy.

5.2.1.5 Comparisons between women <45 years and ≥45 years (Table 2).

Laser treatment was significantly more common among women in the older age group than in the younger age group (24% vs. 13%). Nevertheless, no significant differences regarding margin status were found between the two age groups. On colposcopy, TZ type 3 was significantly more common at first follow–up in the older age group than in the younger age group (67% vs. 36%) and likewise, abnormal cytology was significantly more common at first follow–up in the older age group than in the younger age group (24% vs. 13%). Positive HPV status was significantly more common at first follow–up among women

≥45 years compared with women <45 years (25% vs. 15%). Approximately 12% of women in both age groups had no further follow-up after the first follow-up. No significant differences regarding age and cytology or HPV status were found at second follow-up or at subsequent follow-ups. Recurrent high-grade cervical dysplasia was more often detected among women ≥45 years compared with women aged 30–44 years and women <30 years (11.8% vs. 3.4% vs. 2.1%).

Table 2. Comparisons related to age at conization				
Variable	**< 30 years**	**33–40 years**	**P value**	**≥ 45 years**
Treatment modality			<0.05	
C-LETZ	155	236		58
Laser	131	28		18
Ablation[a]	2	1		0
Margin status[b]			NS	
Both clear	140	190		56
Ectocervical unclear/uncertain	16	21		5
Endocervical unclear/uncertain	13	22		10
Both unclear/uncertain	19	32		5
Cytology at 1st follow-up			<0.05	
NILM	161	234		58
Abnormal	27	31		18
hrHPV at 1st follow-up (Cobas)			<0.05	
Negative	158	228		57
Positive	30	37		19
Recurrent high-grade cervical dysplasia at follow-up			<0.01	
No	184	256		67
Yes	4	9		9
Data were analyzed using two-tailed Pearson's χ2 or Fisher's exact test if any expected cell was <5, one degree of freedom, dichotomized to age ≥45. [a]The three patients who underwent ablation are excluded from the statistical analysis for this variable. [b]Margin status dichotomized to all clear margins vs. one or both margins unclear or uncertain. hrHPV = high-risk human papillomavirus. NILM = Negative for intraepithelial lesions or malignancy.				

5.2.1.6   *Comparisons related to margin status (both margins clear vs. either/both margins unclear/uncertain) (Table 3).*

No significant differences were found regarding treatment modality. However, finding at least one margin that was classified uncertain/unclear occurred significantly more often when the cone was resected in more than one piece as opposed to a single piece. The C-LETZ procedure was associated with a higher risk of resection in multiple pieces compared with other treatment modalities. Cytologic abnormalities were significantly more common at first follow-up among women with at least one unclear/uncertain margin. No significant differences were found for positive HPV status in relation to margin status at first follow-up.

Table 3: Comparisons related to margin status.

Variable	Both margins clear	P value	Endocervical margin unclear/uncertain	Both margins unclear/uncertain	Ectocervical margin unclear/uncertain
Treatment modality		NS			
C–LETZ	329		36	51	33
Laser	54		9	5	9
Ablation[a]	3		0	0	0
Cone pieces[a]		<0.01			
Single	326		40	34	34
Multiple	57		5	22	8
Histology of excised cone		<0.01			
CIN2	112		5	8	8
CIN2/3 or worse	274		40	48	34
Cytology at 1st follow–up		<0.05			
NILM	338		32	44	39
Abnormal	48		13	12	3
HPV at 1st follow–up (Cobas)		NS			
Negative	326		34	43	40
Positive	60		11	13	2
Cytology & HPV (Cobas) at 1st follow–up		NS			
NILM and negative HPV	301		29	37	37
Abnormal cytology &/or positive HPV	85		16	19	5

Pearson's χ2 2-tailed P-values with one degree of freedom with margin status dichotomized to both margins clear vs. one or both margins unclear or uncertain. [a]The three patients who underwent ablation are excluded from the statistical analysis for this variable. CIN = cervical intraepithelial neoplasia. C-LETZ = contoured-loop excision of the transformation zone HPV = high-risk human papillomavirus. NILM = Negative for intraepithelial lesions or malignancy.

5.2.1.7   Multiple logistic regression findings with detected recurrence as the outcome (Table 4).

Table 4 presents three statistically significant models (A, B and C) with 100% of the women included. Model A includes four independent variables, each of which has a significant predictive value for detecting recurrence: 1) age ≥ 45 at conization, 2) one/both unclear or uncertain margins at conization 3) positive HPV at first follow-up (Cobas) and 4) abnormal cytology at first follow–up.

Model B included an additional variable, 5) any diagnosed comorbidity; however, it was not a significant predictor of recurrent high–grade cervical dysplasia. Model C employed a modification of the fifth variable – any diagnosed comorbidity linked to HPV or to CIN progression, but it had no significant predictive value for recurrent high–grade cervical dysplasia either.

In a further multiple logistic regression analysis (after adjusting for age, HPV at first follow-up and abnormal cytology) we found a significantly greater likelihood of detecting recurrent high–grade cervical dysplasia when endocervical (OR=6.2 [95% CI: 1.8-21.4],

p=0.004) or both endo- and ectocervical margins (OR=5.5 [95% CI: 1.6-18.9], p=0.006) were unclear/uncertain, but not when ectocervical margins alone were unclear/uncertain.

Model x^2	Variable	OR	−95% CI	+95% CI	P value
Table 4: Prediction of detected recurrent high-grade cervical dysplasia					
A[a]					
	Age ≥ 45	3.5	1.3	9.9	<0.05
	≥ 1 unclear/uncertain margin	5.3	2	14.2	<0.001
	HPV positive at first follow-up (Cobas)	5.8	2	16.8	<0.01
	Abnormal cytology at first follow-up	3.9	1.4	11	<0.05
B[a]					
	Age ≥ 45	3.4	1.2	9.6	<0.05
	≥ 1 unclear/uncertain margin	5.4	2	14.5	<0.001
	HPV positive at first follow-up (Cobas)	5.8	2	16.7	<0.01
	Abnormal cytology at first follow-up	3.9	1.4	11.2	<0.05
	Any diagnosed comorbidity	1.3	0.4	3.6	NS
C[a]					
	Age ≥ 45	3.4	1.2	9.6	<0.05
	≥ 1 unclear/uncertain margin	5.4	2	14.6	<0.001
	HPV positive at first follow-up (Cobas)	5.8	2	16.8	<0.01
	Abnormal cytology at first follow-up	3.8	1.3	11	<0.05
	Any diagnosed comorbidity linked to HPV or o CIN progression[b]	1.5	0.4	5.8	NS

The data are complete for all predictor variables and outcome: Detected recurrent high-grade CIN (n=22) vs. no detected recurrence (n=507). [a]Model χ2 P<0.0001. [b]Autoimmune disease, human immunodeficiency virus infection, hepatitis, malignancy, diabetes, genetic disorder. CI = confidence interval CIN = cervical intraepithelial neoplasia. HPV = high-risk human papillomavirus. NS = statistically non-significant (P≥0.05). OR = odds ratio.

5.3 Study 3

5.3.1.1 Characteristics of the study sample

Most (79.7%) of the women in this study were <40 years at the time of conization. Laser was the most common treatment modality. Histology showed AIS with coexisting squamous pathology (CIN3 in 76% of cases) in 59.5% of the women and AIS alone in 40.5% of the women. Half of the cohort had unclear/uncertain margins and approximately one-quarter had both margins unclear/uncertain. Information on smoking status was only available in one-third of the women, about 70% of whom had never smoked. Comorbidity was found among two-thirds, 25% of whom had a comorbidity thought to interact with HPV acquisition or CIN progression.

5.3.1.2 Single characteristic data from first follow-up

Just over 50% had their first follow-up within six months post-conization and approximately 85% within one year. The majority had three or more follow-ups. Cytology showed AIS and/or HSIL in five women, while almost 60% were NILM on all post-conization follow-ups. Just over 40% had only negative HPV results, while 20% had at least one

positive HPV test. HPV data were missing for almost 40% of the women, while two or more HPV results were available for 12%. On average, participants were followed for 4.6±3.6 years, with the longest follow-up being 14 years.

5.3.1.3 Analysis in relation to outcomes

Approximately 20% of women underwent reconization and about an equal number underwent hysterectomy. Biopsy results after initial treatment were missing for more than 60% of participants; of the remainder, nearly 17% had no dysplasia and 2% had CIN1. Recurrent AIS alone was found in 8.3% of participants. Two patients were found to have invasive cervical cancer. In all, histologically confirmed recurrence or worse was found in 14.3% and likely recurrence or worse, but without histopathological confirmation, in 3.6% (all of whom had AIS or HSIL on cytology between 2 and 36 months post-conization; all were treated with hysterectomy).

5.3.1.4 Independent variables significantly associated with recurrence

In women with recurrent high-grade cervical dysplasia vs. women without recurrence, no significant differences were found concerning age, comorbidity, treatment modality, or histological findings in the excised cone.

Recurrence was detected more frequently in women with any unclear/uncertain margin(s) (Pearson χ^2=6.7, P=0.01). Significance in relation to recurrent disease was more frequently borderline in women with unclear/uncertain endocervical margins (Fisher's exact test, one tailed P=0.05). However, recurrence was not significantly more frequent among women in whom ectocervical margins alone or both margins were unclear/uncertain.

Among women with known cytology results, at least one abnormal cytology finding post-conization was significantly more likely in those who ultimately suffered recurrent disease (Fisher's exact test P=0.004). Likewise, among women with at least one HPV result, the finding of at least one positive HPV test post-conization was significantly more likely to be found in those who ultimately suffered recurrent disease (Fisher's exact test P=0.001) and they were also significantly more likely to be positive for HPV18 (Fisher's exact test P=0.000). Similarly, among women with two or more HPV results, the finding of at least two positive HPV results post-conization was significantly more likely to occur in those who ultimately suffered recurrent disease (Fisher's exact test P=0.002). Finally, recurrent high-grade cervical dysplasia was significantly more likely to be found in smokers/former smokers (Fisher's exact test P=0.0022).

Table 1 illustrates the sensitivity, specificity, NPV, PPV and overall accuracy for five (A–E) independent variables that were significantly associated (as described above) with recurrent high–grade cervical dysplasia: A) abnormal cytology at follow–up, B) at least one positive HPV finding at follow–up, C) at least two positive HPV findings at follow–up, D) any margin unclear/uncertain and E) current/former smoker. Women who underwent hysterectomy soon after initial conization because of unclear/uncertain margins and women who likely had recurrent disease but lacked histopathological confirmation, were excluded from the analysis. Furthermore, women were excluded from the analysis if data were missing regarding any specific variable (A–E).

The presence of at least one positive HPV result at follow–up had the highest sensitivity for predicting recurrent high–grade cervical dysplasia, while the variable current/former smoker provided the highest specificity for predicting recurrent high–grade cervical dysplasia.

Table 1. Sensitivity, specificity, negative and positive predictive value of significant factors in bivariate analysis in relation to outcome: Histopathologically confirmed recurrent/residual high-grade cervical intraepithelial neoplasia or worse in patients treated by conization for high-grade AIS.

Variable	NPV	PPV	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)
A) Abnormal cytology at follow–up[a]	95.6	30.8	80.0 (44.4-97.5)	70.5 (57.4-81.5)	71.8 (59.9-81.9)
B) ≥1 HPV-positive finding at follow-up[b]	96.8	43.8	87.5 (47.4-99.7)	76.9 (60.7-88.9)	78.7 (64.3-89.3)
C) ≥2 HPV-positive findings at follow-up[c]	94.1	66.7	85.7 (42.1-99.6)	84.2 (60.4-96.6)	84.6 (65.1-95.6)
D) Any margin unclear/uncertain[d]	95.0	26.5	81.8 (48.2-97.7)	60.3 (47.2-72.4)	63.5 (51.5-74.4)
E) Current or former smoker[e]	89.5	83.3	71.4 (29.0-96.3)	94.4 (72.7-99.9)	88.0 (68.8-97.5)

[a]N=71; [b]N=47; [c]N=26; [d]N=74; [e]N=25.. CI = confidence intervals. HPV = high-risk human papillomavirus; NPV = negative predictive value. PPV = positive predictive value.

Table 2 shows four significant multiple logistic regression models (A, B, C and D) with high–grade cervical dysplasia (or worse) as the outcome. In all models (A–D), women who underwent hysterectomy soon after first conization because of unclear/uncertain margins and women with likely recurrent disease without histopathological confirmation, were excluded. Additionally, women were excluded from models A and B, respectively, if they lacked follow–up data for cytology and/or HPV. In model C, women were excluded if they lacked follow–up data for cytology and/or had no more than one positive HPV finding on follow–up.

Table 2. Multiple logistic regression for the outcome: Histopathologically confirmed recurrent/residual high-grade cervical intraepithelial neoplasia or worse in patients treated by conization for high-grade AIS.

Variable	OR	−95% CI	+95% CI	P value
A. Model χ2 =24.0 (P<0.001; N=47)				
Age at conization	1.15	0.97	1.37	NS
Abnormal cytology at follow–up	1.36	0.07	24.9	NS
HPV18–positive finding at follow–up	141	5.2	3.803	<0.005
B. Model χ2 =18.3 (P<0.001; N=47)				
Age at conization	1.19	0.99	1.43	NS
Abnormal cytology at follow–up	4.40	0.47	41.4	NS
≥1 HPV-positive finding at follow-up	47.6	1.77	1.283	<0.02
C. Model χ2 =13.7 (P<0.01; N=26)				
Age at conization	1.21	0.90	1.63	NS
Abnormal cytology at follow–up	2.67	0.18	39.4	NS
≥2 HPV-positive findings at follow-up	89	1.91	4.141	<0.02
D. Model χ2 =8.58 (P<0.02; N=74)				
Age at conization	1.05	0.98	1.13	NS
Any margin unclear/uncertain	7.21	1.34	38.7	<0.02

CI = confidence intervals. HPV = high-risk human papillomavirus. NS = statistically non-significant (P≥0.05). OR = odds ratio.

After adjusting for age at conization and abnormal cytology at follow–up, significant predictors of recurrent high–grade cervical dysplasia were identified in models A–C. A positive HPV18 test was the strongest and most significant predictor (Model A). The presence of ≥1 HPV-positive finding at follow-up was also a predictor of recurrent disease (Model B), as was ≥2 HPV-positive findings at follow-up (Model C). In model D, after adjusting for age at conization, any unclear/uncertain margin was a significant predictor of disease recurrence. However, after adjusting for abnormal cytology at follow–up this association became statistically nonsignificant.

6 Discussion

6.1 Study 1

The recurrence rate in our study was 12%, which is higher than the overall average of approximately 7%, according to a 2017 meta-analysis that included 97 studies (97). Further detail regarding a few key points will help to clarify the discrepancies.

First, we can speculate that the higher recurrence rate observed in our study is the result of longer follow-up time, enabling us to identify additional recurrences that occurred later. The meta-analysis included only five studies with a follow-up time that was longer than 16 years (97). Ding et al. reported a recurrence rate of 6% where the median follow-up time was 13 months (160). However, another study from China, with a median follow-up time of 74.3 months, showed that the five-year risk of recurrence was 14.8%. Approximately 25% of their cohort developed recurrent disease more than five years post-conization (161). Nevertheless, percentages concerning recurrence rates vary; some studies with longer follow-up times reported recurrence rates of 14–18% (162, 163), while others reported very low rates (1.1%) (164).

Second, it has been shown that women with a baseline of CIN3 on conization are at greater risk of recurrence (160). Nevertheless, we found that severity of pathology at baseline was not a significant predictor of recurrence (P = 0.269). One explanation for this may be that our study included a relatively high proportion of women with CIN3, specifically 625 (63.1%) of the 991. Another reason may be that slightly more than 30% of the women in our study demonstrated unclear/uncertain margins, a higher proportion compared with the meta-analysis, where the corresponding figure for incomplete excisions was 23% (97, 165), and much higher than the 10% found by Ding et al. (160).

Our study did find an increased risk of recurrence when margins were involved, which is in line with two large meta-analyses (97, 165). Reich. et al. reported a low recurrence rate (0.35%) in 4417 women with clear margins who were followed for up to 30 years after treatment for CIN2+ (166), while they noted that 84 (22%) of the 390 women treated for CIN3, but for whom excision margins were unclear, developed recurrent CIN3 (167).

Not many studies have analyzed the effect of comorbidity on disease development among women with high-grade cervical dysplasia. Most previous studies have mainly focused on the likelihood of recurrent disease among HIV-positive women (168, 169). One study reported smoking and immunosuppression as risk factors for recurrent disease (160). However, we found that comorbidity alone was a significant factor for predicting recurrent disease. These findings highlight the significance of medical history and comorbidities in risk assessment of women with high-grade cervical dysplasia, as well as the need for stricter post-treatment monitoring in immunosuppressed women.

We found no association between either treatment modality or unclear/uncertain margins and risk of recurrence. A 2013 Cochrane review reported similar findings (149). Because our study was conducted prior to the adoption of hrHPV testing as standard clinical practice, a relatively low percentage (22%) of the women had follow-up data on hrHPV.

When compared with evaluation of biopsy margin status, hrHPV testing has higher sensitivity (91% vs. 56%) and equal specificity (84%) for predicting recurrent disease (97). A 2020 study showed that testing for hrHPV post-conization had high specificity (80%) and sensitivity (88.8%) for detecting recurrence. Furthermore, considering HPV data in relation to margin status and/or cytology did not significantly improve prediction accuracy (96). Ding et al. obtained similar results, in which sensitivity of margin status for predicting recurrence was 53.1%, whereas it was 88.5% for hrHPV; taking margin status into account did not increase prediction accuracy (160). However, in contrast to our study, the above studies did not further subdivide margin status to consider endo- and ectocervical margins separately, as well as together. Therefore, we suggest that combined evaluation of sub-divided margin status and hrHPV status post-conization could be a powerful predictor of treatment failure. This is consistent with another study that showed a PPV of 94% and NPV of 96% when evaluating a combination of endocervical margin status and hrHPV status to predict recurrent disease (170).

The latest international follow-up guidelines from 2019 recommend HPV testing six months post-conization for women with high-grade cervical dysplasia, followed by annual HPV testing until three sequential negative tests are obtained. The recommendation thereafter is for surveillance with HPV testing at three-year intervals for at least 25 years (153). The Swedish national guidelines recommend HPV testing and cytology six months post-conization. If treatment was for AIS, HPV and co-testing are recommended at 6 and 18 months post-conization; if HPV-negative and NILM on cytology after 6 (and 18) months, surveillance with HPV test and cytology are recommended at three-year intervals until "old age" ensues (30).

This study is distinguished from other studies by the long follow-up time, large size, extensive information from medical records and almost complete follow-up data through the linkage to the Swedish National Cervical screening registry. However, data on recurrencies outside the cervix, for example vaginal high-grade dysplasia (172) which can be considered as a recurrent cervical high-grade dysplasia, was limited in NKCx. When this kind of data was found in medical records it was defined as a recurrent disease. Even though these conditions are rare, the actual recurrence rate may be a bit higher than what we have reported. Moreover, we did not have information on reconization that may have occurred outside KUH.

This cohort study only included patients from KUH which may affect the generalizability of our results. For example, 1.7% of the cohort had HIV which is higher compared to the

estimated HIV prevalence in Sweden among the general population, which is 0,08% (173). An interesting observation is that 19 women (approximately 2%) of the cohort was younger than 23 years, meaning that their dysplasia was not detected in the screening program but rather in investigation of symptoms such as coital bleeding. However, the majority of the cohort probably had detected dysplasia in the screening program. Non-attenders are at high risk of developing cervical cancer and there is a risk of missing that patient group in the cohort since they might have developed advanced disease already. Another limitation of this study is the lack information about hrHPV status which was only available for 22% of the cohort.

6.2 Study 2

In all, 22 women (4.1%) in this study experienced recurrent disease. As found by several other investigations, including study 1 (171), margin status and recurrence have a strong association (97, 165). Moreover, we again conclude that recurrence was associated with positive findings in endocervical margins alone or in both margins, but not in positive ectocervical margins alone, all of which agrees with study 1 (171).

Comorbidity was not a significant predictor for recurrent disease in this study, but in some individual cases, autoimmune disorders may have contributed to cervical dysplasia. Autoimmune disorders have previously been linked to increased risk of cervical dysplasia (172). Additionally, in line with a Cochrane review (149) and study 1 (171), treatment modality and initial lesion severity were not found to be associated with increased risk of recurrence.

Also consistent with other studies, we found a significant association between age and recurrent disease (98, 173). Women 45 years or older were at increased risk of recurrence and significantly more often had abnormal cytology and positive HPV findings at first follow-up, compared with the younger age group. Women 45 years or older did not significantly more often have positive margins compared with the younger age group. Nevertheless, the majority of women aged 45 or older with recurrent disease also demonstrated positive margins, which may support the recommendation for more aggressive treatment among non-fertile women (97).

This study identified four independent variables for predicting recurrence. Positive margins in the cone biopsy were the most significant predictor of recurrent high-grade cervical dysplasia, suggesting a need for more frequent post-conization follow-up regardless of HPV status. Moreover, a positive HPV test soon after conization was also a predictor of recurrence. However, a negative HPV test soon after treatment was found in more than 30% of women with recurrent disease. Subsequently, most of these women were found to become HPV-positive during routine screening, emphasizing the

importance of repeated HPV testing. Abnormal cytology soon after conization was also a significant predictor of recurrent disease. Our findings support the Swedish national guidelines, where more intensive follow-up is recommended for women who demonstrate either abnormal cytology or positive HPV results post-conization (30). Lastly, age $\geq$45 years was also a significant predictor of recurrent high-grade cervical dysplasia, which justifies increased scrutiny of women in this age group. Individualized follow-up is crucial to prevent these women at increased risk of recurrence from developing cervical cancer.

This study is distinguished from others by the completeness of its data. HPV and cytology data were available for all patients, as was information regarding comorbidities, histology on cone biopsy, treatment modality and margin status. Almost 100% of patients presented for early follow-up post-conization and subsequent data were available for almost 90% of the cohort. Moreover, duration of follow-up was relatively long at 4-6 years.

However, in the second follow-up, 108 patients were included where three patients were missing cytology results. Thus, 105 cytology results from the second follow-up were available but only 94 HPV results. The discrepancy between cytology- and HPV results was not expected since HPV-test should be done as a reflex-test on LBCs. This indicates that some of the private gynecologist continued to only test for cytology on smears during follow-up post treatment, and were not following the national guidelines. Any potential recurrence among the 10.5% who were missing HPV data in the second follow-up might have been detected and treated earlier if HPV test would have been performed. Data shortcomings concerning persistent HPV infection was another limitation.

6.3 Study 3

This study found that positive hrHPV status, especially HPV18, post-conization is a strong predictor of recurrence in women treated for AIS, underscoring the need for repeated HPV testing following treatment for AIS. Similar results were reported in another study by Costa et al., who showed that HPV testing following treatment for AIS has higher sensitivity for predicting recurrence than cytology (90% vs. 60%, respectively) (174).

According to a 2017 meta-analysis, positive HPV status post-conization as well as incomplete resections can predict recurrent high-grade cervical dysplasia post-conization. A relative increase in risk of recurrence was noted in women treated for glandular pathology compared with those treated for squamous pathology, but no stratified data were provided for women with AIS (97). The risk of recurrent high-grade cervical dysplasia was also higher among women with incomplete resections compared with women whose cone biopsy showed negative margins, which is in line with several other studies (175-177). Nevertheless, risk of recurrence among women treated for AIS was increased even among those with negative margins on biopsy (178-180). In the present

study, 16.6% of all women with recurrence had negative margins. Recurrence rate was not significantly higher among women with AIS and concurrent squamous pathology than in women with AIS alone.

As mentioned above, smoking is a known risk factor for cervical dysplasia and cervical cancer (75, 76). Smoking status may also help predict risk of recurrence in that smokers and former smokers demonstrated significantly higher risk of recurrent high-grade cervical dysplasia compared with never-smokers. A threefold greater risk of recurrence has been reported in smokers treated for high-grade cervical dysplasia compared with never-smokers (181).

Disease recurrence could not be predicted based on either age at conization or abnormal cytology, when each is considered alone. Furthermore, we found no association between comorbidity and recurrence.

Strengths of this study include long follow-up time of up to fourteen years and its linkage to the Swedish National Cervical Screening Registry, which contains complete cervical screening data.

One limitation of this study was lack of HPV data. Just under 40% of all participants lacked all follow-up HPV data, while almost 30% had only one follow-up HPV test. However, the majority had at least two clinical post-conization follow-ups, mainly entailing cytological examination. One explanation, at least pertaining to early cases, may be that clinical guidelines did not yet recommend HPV testing. Another possible reason may be that some women were followed by private clinicians who either did not report HPV results, or simply did not follow the guidelines at the time.

This lack of follow-up conflicts with international guidelines. The recommended follow-up after treatment for AIS is HPV testing and cytology every six months for the first three years, and then annually for at least two years. If results are consistently negative for HPV and show NILM on cytology for five years, follow-up including HPV testing and cytology should continue lifelong at three-year intervals. However, these recommendations are specific for women who may wish to become pregnant in the future and who undergo conservative treatment, such as conization. For women who do not wish to become pregnant or are beyond childbearing age, hysterectomy is the recommended treatment of choice for AIS. Recommended post-hysterectomy surveillance should include vaginal examinations and HPV testing for 25 years. (57). The relatively small number of women in this study may also be considered a limitation.

7 Conclusions

7.1   Study 1

Women with incomplete resection were at increased risk of recurrent high-grade cervical dysplasia, especially if both endocervical and ectocervical margins or endocervical margins alone were positive, but not in cases of positive ectocervical margins alone. Positive hrHPV findings post-conization further increase risk of recurrence among these women. In addition, comorbidity alone is a predictor of recurrence. Therefore, subdivision of margin status combined with hrHPV testing and attention to comorbidities may help to identify women who are at risk of recurrent disease and in need of retreatment.

7.2   Study 2

In conclusion, positive margins on cone biopsy, age ≥ 45 years, positive hrHPV findings at first follow-up and abnormal cytology at first follow-up were identified as significant independent risk factors for recurrent disease. Furthermore, persistent hrHPV infection was also found to have a significant association with recurrent disease. Women with incomplete resections, abnormal cytology and/or positive hrHPV findings post-conization require more intense follow-up; special attention is warranted for women 45 years or older.

7.3   Study 3

To sum up, hrHPV testing following treatment for AIS yielded the highest sensitivity for detection of treatment failure. Status as a smoker or former smoker generated the highest specificity for recurrence. The strong predictive value of HPV, especially HPV18, in relation to recurrence indicates that HPV testing is essential in post-treatment follow-up for AIS. In addition, it is important to focus on smoking and to encourage long-term follow-up to better protect these women who are at higher risk of progression to invasive cervical cancer.

8 Points of perspective

Whether or not HPV vaccination can lower the risk of recurrent disease is still a matter of debate. A 2013 retrospective study found a lower rate of recurrent high-grade cervical dysplasia among women who had received the quadrivalent HPV vaccine post-conization than among those who did not (2.5% vs 7.2%) (182). Gheraldi et al. found in the SPERANZA project (a non-randomized prospective study) that the risk of recurrence within 4 years post-conization was significantly reduced among women in the vaccinated group compared with the non-vaccinated group (1.2% vs 6.4%, P = 0.0112) (183). However, several other studies found no evidence that HPV vaccination contributes to HPV clearance or reduces risk of recurrence (184, 185). A large 2022 meta-analysis which included 22 studies found that HPV vaccination may reduce risk of recurrence post-conization, especially when HPV16 or 18 are involved. However, the data were inconclusive and larger randomized controlled trials are needed to determine the efficacy of perioperative HPV vaccination (186). Two other meta-analyses from 2020 and 2021 showed similar results (187, 188).

Currently, there is one randomized controlled study ongoing; the NOVEL-trial, with the aim to investigate if HPV vaccine given at the same time as conization can lead to reduced risk of recurrent disease and cervical cancer development compared to conization alone. One-thousand women who are not pregnant, aged between 18-55 years and treated for high-grade cervical dysplasia will be included in the study. They will be followed up for up to 2,5 years. Results are expected in 3-4 years, at the earliest (192).

According to Swedish national guidelines, HPV vaccination may be recommended for women post-conization to help prevent recurrence and the need for further surgery, which may be associated with higher risk of pre-term birth (30).

Under circumstances like the COVID-19 pandemic, self-sampled HPV testing (ssHPV) is reliable (156, 189-191) and becomes a good alternative to screening sampling by a clinician (192). In late 2022, the Swedish guidelines were modified to recommend HPV sampling as the primary test for all women in the national screening program and ssHPV was incorporated as an alternative mainly for non-attenders. However, future studies are needed to evaluate the use of ssHPV for post-conization follow-up, especially in women who have been treated for AIS.

Conization may be associated with unfavorable reproductive morbidity in subsequent pregnancies. However, results and conclusions from published studies are contradictory, which may be due to weaknesses such as small sample size and short follow-up time. It is unlikely that a randomized controlled trial comparing pregnancy outcomes among women who have undergone conization with those who have not will ever be conducted. Since untreated precancerous lesions may develop into cervical cancer, such a study

would be unethical. To the best of our knowledge, no observational prospective study has followed the same cohort for a period of decades to investigate the risk of recurrence as correlated to age, whether conization was radical, cone size, HPV status and obstetrical outcome. A Swedish retrospective population-based register study including over 1 million women found that HPV infection diagnosed shortly before or during pregnancy increased the risk of obstetrical complications, including pre-term delivery and neonatal mortality; the risk was even higher among women who had undergone treatment for cervical dysplasia (193). Finding the optimal strategy to protect women with high-grade cervical dysplasia from obstetrical complications, while also protecting them from cervical cancer, should be a top priority. Obstetrical risk versus treatment benefit requires further study so as to clarify the uncertainties related to this issue and to facilitate clinical management for this patient group.

9 Acknowledgements

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