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OPTIMIZING CERVICAL CANCER PREVENTION THROUGH SCREENING AND HPV VACCINATION

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Stockholm 2015

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Published by Karolinska Institutet.
Printed by AJ E-print AB
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ISBN 978-91-7549-974-1

Karolinska Institutet

Institutionen för medicinsk epidemiologi och biostatistik

Optimizing cervical cancer prevention through screening and HPV vaccination

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i hörsal Petrén, Nobels väg 12B, Karolinska Institutet, Solna

Måndagen den 15 juni 2015, kl 09.00

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Stockholm 2015

To my family

At any rate, that is happiness; to be dissolved into something complete and great.

- *My Antonia, Willa Cather*

ABSTRACT

Effective primary and secondary prevention tools exist for cervical cancer in the form of human papillomavirus (HPV) vaccines and cervical screening. In order to maximize the impact of prevention strategies in Sweden and European countries, this thesis sought to investigate the long-term effectiveness of different screening strategies and the long-term risk associated with HPV infections, the organization and quality of existing screening programs, and the effectiveness of alternative vaccination strategies.

HPV-based screening has been evaluated using intermediate outcomes while its effectiveness against cancer had not been fully examined. In **Study I**, the European randomized controlled trials (RCT) of screening methods were pooled to investigate the relative efficacy of HPV-based versus cytology-based screening for the prevention of invasive cervical cancer. We found that HPV-based screening provides 60-70% greater protection against invasive cervical cancer compared to cytology-based screening.

To address the issue of determining intervals for HPV-based screening and to investigate concerns regarding overdiagnosis with HPV-based screening, a long-term follow-up of the Swedescreen RCT was completed in **Study II**. The longitudinal performance of cytology- and HPV-based screening was explored and the sensitivity for cervical intraepithelial neoplasia grade 2 or worse (CIN2+) of HPV testing at 5 years of follow-up was similar to that of cytology testing at 3 years. Over 13 years of follow-up, we found that the increased sensitivity of HPV screening for CIN2+ reflects earlier diagnosis rather than overdiagnosis and low long-term risks among HPV negative women suggest that extending screening intervals with HPV-based screening would be possible.

The incidence of low-grade cervical lesions is increasing in Sweden. Low-grade lesions require follow-up, creating a burden to the woman and the healthcare system. Examining the long-term HPV-type-specific risk for atypical squamous cells of undetermined significance (ASCUS), low grade squamous intraepithelial lesions (LSIL) and cervical intraepithelial neoplasia grade 1 (CIN1) is of interest to inform screening and vaccination programs. In **Study III**, we investigated the long-term type-specific absolute risk, population attributable proportion, and incidence rate ratios for ASCUS/LSIL by HPV type. The type-specific IRRs for ASCUS/LSIL were high in the first screening round but decreased over subsequent screening rounds. Type 16 contributed to the greatest proportion of low-grade lesions in the population followed by type 31. Most lesions were caused by new infections and found in the first screening round.

Organized, population-based screening with quality assurance (QA) at all levels is recommended by the European Commission to ensure equity and cost-effectiveness of programs. Significant differences in cervical cancer incidence and mortality exist between European countries. In **Study IV**, a comprehensive questionnaire was developed and circulated among EU/EFTA countries to map current organization of programs and quality assurance efforts to understand prevention activities and inform future guidelines. The findings show that organized efforts for QA, monitoring and evaluation differed between and within countries, making it difficult to compare program effectiveness.

HPV vaccination is underway in most European countries but efforts to organize and standardize vaccination program monitoring and evaluation are limited. Using the same questionnaire as in Study IV, we collected detailed information on HPV vaccination programs in EU/EFTA countries for **Study V**. Our findings suggest that the monitoring being performed varies across programs with regard to level of detail and the organization and quality of programs differ. There was a strong interest in the survey which affirms the significance of the issues addressed and the importance of continuing to evaluate program development and strengthen surveillance of vaccination program efforts.

Since the introduction of HPV vaccination, vaccine prices have decreased significantly making upscaling of vaccination efforts more attractive. Specifically, questions have arisen regarding vaccination of older girls and extending the vaccination program to boys. Using a dynamic transmission model, in **Study VI** we compared different vaccination strategies and assessed the resilience of the vaccination program to a reduction in coverage. We found that vaccination strategies including an extended catch-up of women and introducing male vaccination may accelerate the prevalence reduction of vaccine HPV types among women. Further, including males in routine vaccination improved the resilience of vaccination programs.

Taken together, the results of these studies seek to add evidence for the incremental optimization of prevention programs. The challenge will be translating research findings into practice and ensuring that programs have the tools they need to effectively monitor and evaluate changes.

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III. Elfstrom KM, Smelov V, Johansson AL, Eklund C, Naucler P, Arnheim-Dahlstrom L, et al. Long-term HPV type-specific risks for ASCUS and LSIL: A 14-year follow-up of a randomized primary HPV screening trial. Int J Cancer. 2014.

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CONTENTS

LIST OF ABBREVIATIONS

AR	Absolute risk
ASCUS	Atypical squamous cells of undetermined significance
CER	Comparative effectiveness research
CIN	Cervical intraepithelial neoplasia
CIN2+	Cervical intraepithelial neoplasia grade 2 or worse
CIN3+	Cervical intraepithelial neoplasia grade 3 or worse
EMA	European Medicines Agency
EFTA	European Free Trade Agreement
EU	European Union
FIGO	International Federation of Gynecology and Obstetrics
HPV	Human papillomavirus
IARC	International Agency for Research on Cancer
IRR	Incidence rate ratio
LSIL	Low grade squamous intraepithelial lesion
MSM	Men who have sex with men
MSW	Men who have sex with women
NKCx	Swedish National Cervical Screening Registry
NPV	Negative predictive value
PAR	Population attributable proportion
PCR	Polymerase chain reaction
PPV	Positive predictive value
QA	Quality assurance
QC	Quality control
RCT	Randomized controlled trial
RHS	Randomized health services study
SCR	Swedish Cancer Registry
SNOMED	Systematized Nomenclature of Medicine
STI	Sexually transmitted infection
TPR	Swedish Total Population Registry
WHO	World Health Organization

1 INTRODUCTION

Screening for cervical cancer was implemented before we knew what caused it. In the late 1920s, with a pioneering research spirit and somewhat unorthodox methods, Georgios Papanikolaou discovered that smears taken from the vaginal tract could be analyzed for cellular changes indicating cancer. Early epidemiological studies examining risk factors for female cancers found that cervical cancer incidence was much lower among nuns as compared to married women, suggesting that cervical cancer was caused by some aspect of the "coital experience" (1). Using, and further refining Dr. Papanikolaou's "Pap" smear, cervical screening programs have been developed to identify abnormalities and remove them to prevent the development of invasive cancer. In the 1970s, Harald zur Hausen published a hypothesis proposing that human papillomavirus (HPV) could play a role in the development of cervical cancer (2), an etiological link he later demonstrated in the 1980s through identifying HPV types 16 and 18 in cervical cancers (3). HPV has since been categorized as a necessary, but not sufficient cause of cervical cancer and screening tests for the virus developed (4).

Cervical screening exists to varying degrees in most high income countries and vaccination against HPV has been introduced in a variety of settings using a range of implementation schemes. With the implementation of cervical screening programs, cancer incidence has decreased and with the advent of HPV vaccination, protection against the most oncogenic HPV types is possible. We have made tremendous progress in understanding the etiology and natural history of cervical cancer. Now, we have a unique opportunity in front of us to maximize the potential of these prevention strategies. However, several questions remain – what screening tests and intervals should be used? What is the right balance of test effectiveness and acceptability while ensuring overall safety? How should programs be organized and what methods should be used to ensure their quality? What is the optimal target age(s) for HPV vaccination and should we extend vaccination to boys?

This thesis seeks to address some of these remaining questions. The background section focuses on aspects of cervical cancer and HPV research that pertain to the work covered in this thesis, highlighting some of the complexities of this research field and providing context for the studies included. In the concluding thoughts, applications of the findings and future directions are considered.

2 BACKGROUND

2.1 HUMAN PAPILLOMAVIRUS

Currently, 202 human papillomavirus (HPV) types have been identified (5) with 2 more types under investigation, of which 40 are known to be sexually transmitted (6). The International Agency for Research on Cancer (IARC) has classified 13 of these types as oncogenic or high risk (HR) types (7). Types 16 and 18 are particularly notable in this group. They are responsible for approximately 70% of cervical cancer cases in the world (8), with some variation between regions. While less threatening from a cancer risk perspective, non-high risk (non-HR) HPV types 6 and 11 cause approximately 90% of condyloma (genital warts) (9). Humans can be infected with one or multiple types at the same time. There is conflicting evidence on whether specific types tend to appear together (clustering) in multiple infections. While some evidence suggests that types do not appear more frequently together than they would have by chance (10, 11), other evidence suggests that there is clustering of types (12) and evidence for potential type-competition (13).

HPV is the most common sexually transmitted infection (STI) and the risk of acquiring HPV from a first partner is high, especially if the partner is sexually experienced (14). The transmission probability of HPV 16 has been shown to be 40-60% per partnership (15, 16). In women without cervical abnormalities, worldwide prevalence of HPV is estimated to be 11-12%, with a prevalence over 30% in the Caribbean and Eastern Africa and below 5% in Northern American and Western Asia (17). Prevalence usually peaks among 20-30 year olds, again with variation between regions and related, in part, to sexual behavior and screening availability (18). Worldwide, prevalence among women under 25 has been estimated to be 24% and for women ages 25-34 prevalence was 14% according to a large meta-analysis of data on women without cervical abnormalities (19). Prevalence estimates for males come primarily from smaller cohort studies. Among young male Danish conscripts ages 18-29, prevalence was 34% (20). In the Human Papillomavirus in Men (HIM) Study prevalence was higher (36% to 51%), peaking again between ages 18-34 but with differences by sexual orientation (21).

Unlike other STIs that are transmitted through bodily fluids, HPV is transmitted by skin to skin contact. Therefore, condoms can reduce the risk of transmission but do not fully prevent transmission (22). More recent studies examining oral prevalence of HPV suggest that HPV can be transmitted through oral-oral as well as oral-genital routes (23). Although rare, HPV can also be vertically transmitted from mother to child, with vaginal delivery being more risky than caesarean section (24). Risk factors for HPV are number of sexual partners, age at first intercourse, oral contraceptive use, infection with other STIs, immunosuppressive conditions (e.g. HIV), and smoking (25).

Most HPV infections are transient with nearly 90% of infections clearing within 2 years (26) but type-specific clearance rates may differ, as demonstrated by Finnish modeling results (27, 28). The role that natural immunity may play in protecting individuals against reinfection with the same type has been difficult to study as separating new infections with the same type

from infections that are periodically undetectable is challenging from a methodological standpoint (29). However, evidence from a long-term follow-up of a Canadian cohort showed that detection of subsequent HPV infections of the same or a different type was associated with sexual activity, indicating that natural immunity did not provide protection against reinfection (29). Those infections that persist carry a higher risk for developing into precancerous lesions or cancer (30, 31).

Nearly all cervical cancer cases are caused by HPV. Evidence has accumulated rapidly in recent years suggesting a strong link between HPV and other anogenital cancers as well as certain oral pharyngeal cancers in the base of the tongue and tonsils. Recent estimates suggest that 88% of anal cancers are HPV positive (32), 50% of penile cancers, 43% of vulvar cancers, and 70% of vaginal cancers (17). Estimates for the proportion of cancers of the oropharynx that are HPV positive range from 17% to 56% depending on histology and region of the world (17).

2.2 CERVICAL CANCER

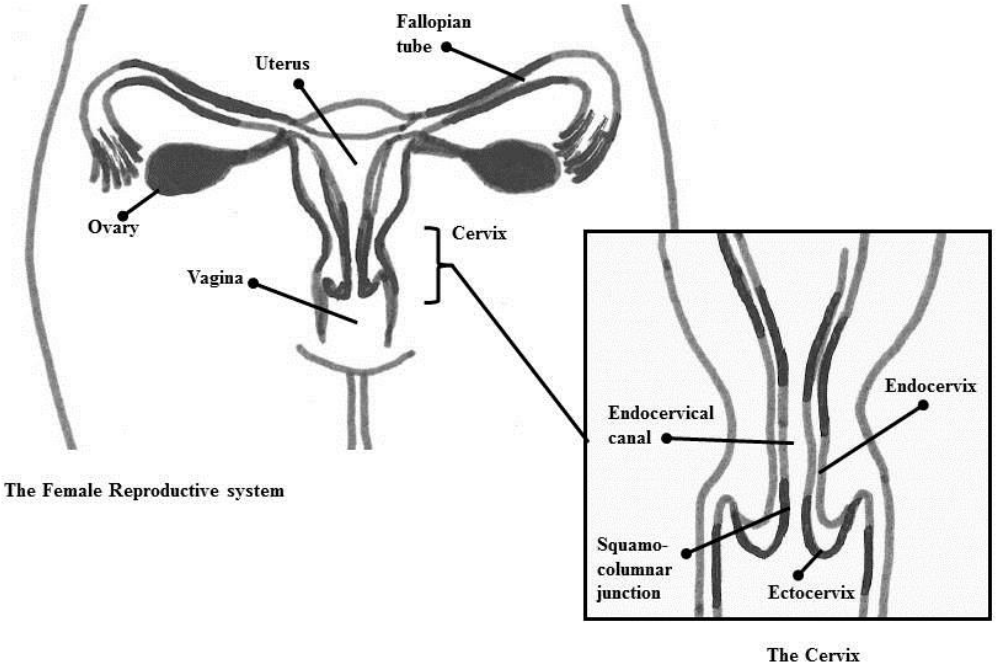
Cervical cancer is the third most common cancer among women worldwide and the second most common cancer among women of reproductive age in Europe (33). In the European region, an estimated 54,000 women are diagnosed with cervical cancer and 25,000 women die from the disease each year (34). Country specific age-standardized incidence rates of cervical cancer vary across the European region from 2.1 to 23.9 per 100,000 women per year (34) and mortality rates vary from 1.1 to 13.7 (35). In Sweden, the age-standardized incidence of cervical cancer was 7.4 per 100,000 women per year with 452 new cases and 178 deaths according to 2008 data (34). Incidence of cervical cancer in Sweden peaks around age 35 with a second peak at age 80 (36). Some evidence suggests that cancers appearing in the younger ages are more likely to be positive for a HR HPV type while cancers appearing among older women can be positive for non-HR types (37, 38).

Squamous cell carcinoma (SCC) and adenocarcinoma represent the two most common histological types of cervical cancer. A nation-wide audit of cervical cancer cases in Sweden showed that 75% of cases were SCC and 20% were adenocarcinoma; other histological types including adenosquamous carcinoma, small cell carcinoma, neuroendocrine tumor, and poorly differentiated histological types were much less common (39). Squamous cell carcinoma originates in the squamo-columnar junction, also known as the transformation zone, whereas adenocarcinoma originates in the glands of the cervix, higher up in the endocervical canal (Figure 1). FIGO (International Federation of Gynecology and Obstetrics) staging is used to classify the extent of tumor invasion. Staging at diagnosis is a significant predictor of cure prognosis (40).

Precursor lesions have been identified and classified for squamous cell carcinoma and adenocarcinoma (to a somewhat lesser extent). Broadly, precursor lesions are identified first in cytology through cervical screening and divided into low- and high-grade lesions. Actual nomenclature used to categorize diagnoses differs across countries. High grade lesions are typically referred directly to colposcopy, a closer examination of the cervix, and biopsy to

determine need for treatment whereas low-grade lesions may be monitored with repeat-testing either with cytology or HPV testing before referring to colposcopy.

Figure 1. Anatomy of the female reproductive system and the cervix



As lesion severity increases, the proportion of lesions that are HPV positive increases and the HPV types represented in the lesion change usually with a greater diversity of types in the low-grade lesions and a dominance of types 16 and 18 in higher grade lesions (41). Low-grade lesions are often considered to be just signs of an on-going HPV infection (41). High grade lesions are typically the result of a persistent HPV infection and can take several years to develop while invasive cancer is the result of a non-regressive lesion and can take 10 years to develop (26). Lesions can regress but rates may differ by age and severity of lesion; higher grade lesions typically have a lower probability of regressing (26, 42). With regard to the effect of age, results from one modelling study show that 84% of lesions will regress in women under 34 compared to 40% in women over the age of 34 (43).

Risk factors for cervical cancer are, in essence, the same as risk factors for contracting an HPV infection. Early age at sexual debut, as a proxy for first exposure to HPV, seems to have a particular impact on risk for cervical cancer (44). Other risk factors parallel risk factors for HPV infection and include: parity, lifetime number of sexual partners (45), smoking (46, 47) hormonal contraception (48), and infection with other sexually transmitted infections (49).

2.3 PREVENTION

Efforts to prevent disease can be implemented at the primary, secondary, and tertiary levels. Primary prevention concerns itself with eliminating risk factors or increasing individuals' resistance to disease before a disease can occur. Vaccination against HPV is an example of primary prevention for cervical cancer where vaccination reduces the likelihood that individuals will become infected with specific HR HPV types, in turn eliminating their risk for cervical pre-cancer and cancer associated with those vaccine types. Secondary prevention concerns itself with detecting disease in the preclinical phase before symptoms appear, and aims to treat early and reduce the chance that individuals experience lasting morbidity. Screening is a form of secondary prevention with the aim of early detection and treatment of cancer. Tertiary prevention is outside the scope of this thesis, but worth mentioning as it is what we seek to avoid through screening for early precursors: the treatment of clinical disease to prevent death or complications (50).

With the advent of HPV vaccination, effective primary prevention of cervical cancer was made possible. Despite the progress with establishing vaccination programs and increasing vaccination coverage, secondary prevention through screening will remain relevant for several generations to come as non-vaccinated women age out of the screening ages and since the current vaccines do not include protection against all oncogenic types of HPV. Working towards integration of prevention activities will be critical.

2.4 PRINCIPLES OF SCREENING

By definition, screening is applied to a population and requires the testing of healthy (asymptomatic) individuals to identify those at risk for developing disease. Screening is:

> "The presumptive identification of unrecognized disease or defect by the application of tests, examinations or other procedures which can be applied rapidly. Screening tests sort out apparently well persons who probably have a disease from those who probably do not. A screening test is not intended to be diagnostic. Persons with positive or suspicious findings must be referred to their physicians for diagnosis and necessary treatment"
>
> *- From the CC1 Conference on Preventive Aspects of Chronic Disease 1951, as quoted in the Wilson Criteria (51)*

In the late 1960s, Wilson and Jungner developed a comprehensive list of criteria for screening. These form the basis for evaluating screening methods and implementation and can serve as a guide when considering changes to existing screening efforts or introducing new programs.

Table 1. Wilson Criteria for Screening (51)

1	The condition sought should be an important health program
2	There should be an accepted treatment for patients with recognizable disease
3	Facilities for diagnosis and treatment should be available
4	There should be a recognizable latent or early symptomatic stage
5	There should be a suitable test or examination
6	The test should be acceptable to the population
7	The natural history of the condition, including development from latent to declared disease, should be adequately understood
8	There should be an agreed policy on whom to treat as patients
9	The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole
10	Case-finding should be a continuing process and not a "once and for all" project.

The Wilson criteria continue to be used and have been further expanded for evaluating the cost-effectiveness of screening (52).

Cervical screening occurs to some extent in most high-income countries; however, the mode of implementation differs. Broadly, screening approaches can be divided into organized and opportunistic screening. Opportunistic cervical screening occurs in settings where screening facilities exist but women themselves must take the initiative to attend or may be reminded to attend by their primary care provider. In an organized screening setting, programs provide for a national or regional team responsible for implementation and require providers to follow guidelines, rules, or standard operating procedures. Organized programs define a quality assurance structure and mandate supervision and monitoring of the screening process. To evaluate impact, organized programs also require ascertainment of the population disease burden. Organized, population-based programs identify and personally invite each eligible person in the target population to attend a given round of screening (53). Both organized and opportunistic screening can achieve cancer incidence reduction (54-56); however, organized screening programs have been more effective than opportunistic programs as they can reach a greater proportion of the target population and provide more equitable care (57). Furthermore, offering screening within the context of an organized program can achieve greater cost-effectiveness, encouraging tests that are taken at an appropriate interval to avoid overscreening and triage strategies that are applied effectively.

European guidelines for quality assurance in cervical screening were first published in 1993 and defined principles for organizing, monitoring, and ensuring quality of screening (58). Ten years later, a recommendation of the Council of the European Union prioritized the implementation of screening programs in EU member-states (59). In 2008, updated European guidelines for quality assurance in cervical screening were released to reflect advances in screening technologies and prevention strategies (53). Often, countries work with layers of

recommendations using national guidelines that build on international recommendations. The EU recommendations apply to all member states, but actual implementation is tailored to individual country settings and existing infrastructures.

Simplistically, catching a cancer early can be viewed as a purely positive result of screening. However, trade-offs inherent in early diagnosis must be considered. Specifically, there is the risk of overdiagnosis, identifying a cancer that would have otherwise never progressed to being symptomatic or to cause death (60). Usually this is the result of either the cancer regressing or growing so slowly that the individual dies of a competing cause (60). Overdiagnosis causes significant distress to the individual and potentially unnecessary procedures that can be invasive and incur lasting side effects, depending on the cancer type and available treatment options. It can be measured at the population level by examining the excess incidence with screening compared to the incidence in the absence of screening (61). In the context of cervical cancer, there has been an increasing concern about the overdiagnosis of precancerous lesions, lesions that would regress and never result in invasive cancer with switches to more sensitive testing methods such as HPV-based screening (62, 63). This is of concern especially among women who want to have children and have been diagnosed with high grade precancerous lesions or early stage cancer. Studies have shown that treatment can increase the risk of miscarriage in the second trimester (64).

2.5 SCREENING METHODS

Screening methods should be evaluated based on their level of reliability and variation within the method and between observers. Furthermore, the choice of test should balance complexity and accuracy with speed and cost (51). There are a variety of cervical screening tests in use which require varying degrees of laboratory infrastructure and personnel skills to implement. The most common tests used in screening programs in high-resource settings are cervical cytology and HPV DNA testing. While cytological screening has reduced the incidence of cervical cancer, the sensitivity is moderate (between 50 – 75%) and can be variable depending on the quality of the sample taken and the sample reader (53, 65). Given concerns about lower sensitivity with cytology-based screening and the etiological link between HPV and cervical cancer, HPV DNA detection as part of primary screening and/or triage has come into favor and has been implemented as a primary screening technique in some European countries (53, 65-68).

Cervical cytology is based on the examination of exfoliated cells from the transformation zone of the cervix and excretions from the endocervical canal collected with a specially designed spatula or brush. This material can either be smeared and fixed directly onto a glass slide (conventional cytology) or released into a vial of fluid (liquid based cytology (LBC)). LBC samples are then filtered: the cellular material is fixed to a glass and interpreted and the liquid can be saved for further analysis, such as HPV DNA testing (69). Comparisons of conventional and liquid based cytology have shown that sample adequacy is often higher in LBC samples (70). This finding, coupled with the ability to reuse the sample for further analysis which eliminates the need to call the woman in for further testing has led

to a switch to LBC in many programs (71). Cervical cytology detects whether there is evidence of cellular changes indicating pre-cancer or cancer.

HPV DNA testing employs the same sort of sample collection but the material is analyzed for presence of HPV DNA instead. Compared to cytology, HPV tests typically have better sensitivity but lower specificity when examining one round of testing. HPV DNA tests can be broadly categorized into methods in which the DNA is amplified (target amplification by polymerase chain reaction (PCR) or signal amplification such as in the hybrid capture 2 test (HC2)) or non-amplified methods (which use nucleic acid probes). HPV tests can also be serology based. According to the Meijer Criteria, key requirements for a HPV test to be used as a primary screening test include high sensitivity and specificity and intra- and inter-laboratory reproducibility (72). Evidence from European screening studies has shown that HC2 and GP5+/6+ PCR meet these requirements (73). The FDA approved the cobas HPV test which identifies types 16 and 18 separately and 12 other HR HPV types together for use as a primary screening test in 2014 (74).

2.6 MEASURING SCREENING TEST PERFORMANCE

Screening test performance can be measured by calculating the sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV). These test characteristics are used to evaluate binary outcomes or continuous outcomes dichotomized at a meaningful cut-point and provide estimates of the test's ability to identify positive and negative test results correctly against a gold standard or the true disease status. Sensitivity measures the ability of the test to correctly identify those who have the disease or condition of interest and is calculated as the number true positives detected by the test over the total number of true positives. Conversely, specificity measures the ability of the test to correctly identify those who are disease-free. It is calculated as the number of disease-free individuals correctly identified by the test divided by the total number of disease-free individuals. Sensitivity and specificity are measures of the test's ability to correctly identify diseased and non-diseased individuals (50).

Another important aspect of a test's performance has to do with how predictive the test results are. In other words, if an individual tests positive, what is the probability that the individual is actually diseased and vice versa? The NPV and PPV of a test give us a sense of how predictive the test is. The NPV is calculated by taking the number of correctly identified disease-free individuals and dividing by the total number of test-negatives identified (in other words, dividing by the sum of the true and false negatives). The PPV is calculated by taking the number of true positives identified by the test and dividing by the total number of positives identified by the test (50). The calculations are outlined in Figure 2.

Ideally, screening tests would be able to perfectly discriminate between those with likely disease and those without; however, in reality this rarely happens. When evaluating a test's performance, no single measure can be taken in isolation. For example, despite having a high sensitivity, a test can still have a low PPV which would cause many individuals to be false-positive and referred for further follow-up unnecessarily. If the follow-up for a test-positive individual is invasive and runs the risk of significant side effects, then a low PPV

would be particularly concerning. When making decisions about which test(s) to use and in what order, trade-offs must be considered and the test chosen should be a reflection of a carefully balancing costs, healthcare burden, burden to the patient, and efficiency.

Figure 2. Calculating sensitivity, specificity, NPV, and PPV

Test results	Truth or results of gold standard		Total	Calculations
	Positive	Negative		
Positive	True positives (TP) – diseased and tested positive	False positives (FP) – disease-free but tested positive	Sum of all test-positive (TP+FP)	PPV = $\frac{TP}{TP + FP}$
Negative	False negatives (FN) – diseased but tested negative	True negatives (TN) – disease-free and tested negative	Sum of all test-negative (FN+TN)	NPV = $\frac{TN}{FN + TN}$
Total	Sum of all truly diseased (TP+FN)	Sum of all truly disease-free (FP+TN)		
Calculations	Sensitivity = $\frac{TP}{TP + FN}$	Specificity = $\frac{TN}{FP + TN}$		

*Adapted from the Wilson criteria (51) and Gordis' Epidemiology (50)

So far, characteristics have been described as they are calculated with cross-sectional data referring to one visit – comparing one test with the "truth", a gold standard test, or the comparison test of interest when both test results were temporally close together. In screening, we often want to know how tests perform over a longer period of time so that we can make decisions about the safety and effectiveness of different screening intervals with different tests. Thus, calculation of longitudinal test characteristics has been proposed as a key screening test performance indicator (75), more on this will follow in the methods section, under Study II.

In the case of cervical cancer screening, cervical cytology has been used as the de facto gold standard, or comparison test when evaluating new screening methods. As such, issues of how good the gold standard truly is arise since cytology has not been studied in a randomized fashion and at this point, comparing cytology to no screening raises ethical concerns. The performance of cytology is a major point of discussion currently and at the heart of the analysis presented in Studies I and II.

2.7 QUALITY ASSURANCE

In order to achieve health gains, programs need to have systems and structures in place to be able to thoroughly evaluate whether the screening program is performing according to guidelines and is achieving the expected effect. Quality assurance consists of the management and coordination of the program throughout all levels of the screening process, invitations to

screening, testing, diagnosis, and follow-up of screen-test positives, to ensure that the program performs adequately and provides services that are effective and in-line with program standards (53).

The European Guidelines for cervical screening outline specifics for designing, implementing, and monitoring the performance of programs. In the 2nd edition of the guidelines, organization of screening, monitoring and evaluation, methods for diagnosis and treatment, and laboratory guidelines for cytology and histology are outlined. Instructions for carrying out audits of cervical cancer cases are included and point to the importance of evaluating both the process of screening – whether guidelines are being followed – as well as the impact of screening, are we preventing cases as we intended? Additionally, the guidelines provide suggestions for diagnosis terminology, methods for calculating key performance indicators. The guidelines introduce HPV testing as a primary screening method and mention vaccination as a new prevention tool. Taken together, the guidelines are detailed, specific, and comprehensive in their recommendations. They build on an extensive review of the literature and expert evaluation of existing evidence.

Incremental revision of screening programs in the form of sequentially optimizing with new strategies is ideal as each change can be monitored and evaluated for logistical ease and health impact. As healthcare programs increasingly face budgetary trade-offs, evaluating strategies from a health economics perspective will be critical and evaluating new strategies in comparative health effectiveness studies could be a way forward.

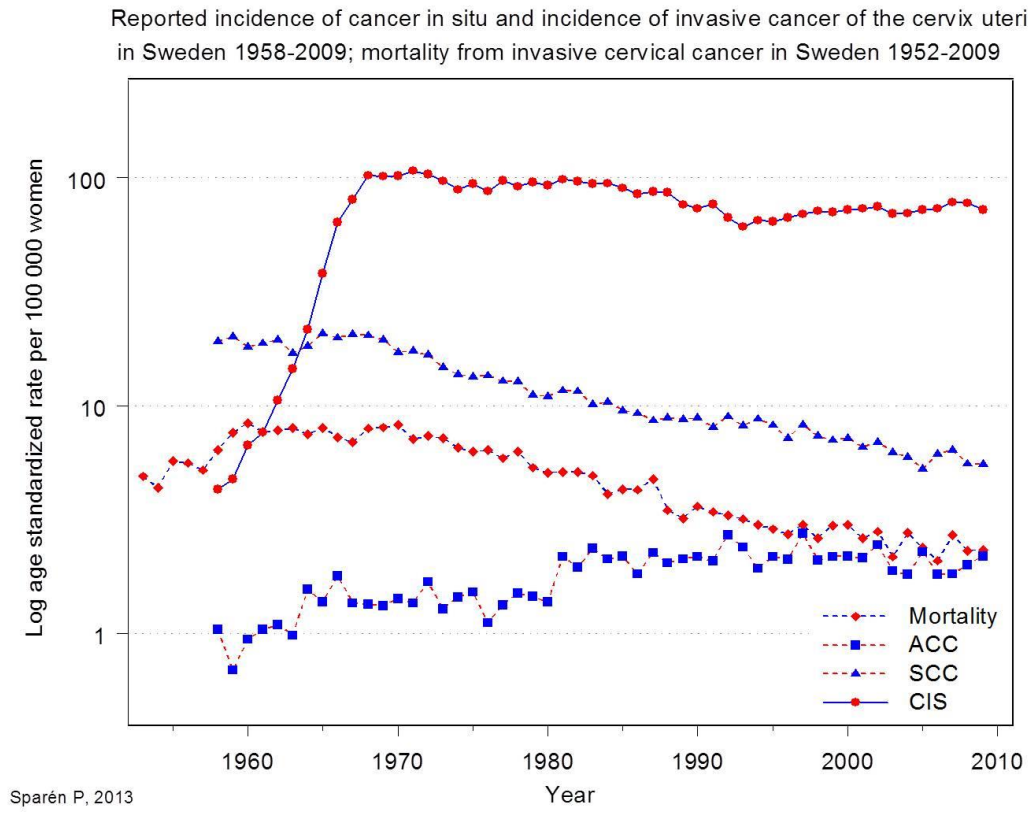
2.8 CERVICAL SCREENING IN SWEDEN

Organized cervical screening began in the 1960s in Sweden and was nationwide by the 1970s. Since 1998, women ages 23-50 have been invited to screening every 3 years and women ages 50-60 have been invited every 5 years, which translates into 12 screening tests per woman per lifetime. Invitations are sent once 3 or 5 years have passed since the last screening test recorded. Therefore, actual attendance is usually measured within 3.5 or 5.5 years. If a woman does not attend following her invitation, a reminder is sent each year until she attends or actively opts out of the invitational system. Opportunistic tests, taken outside the organized program, are integrated into the call and recall system and delay the next organized invitation until the age-specific interval has passed with the goal of reducing over-screening, currently at around 10%. In 2013, 69% of screening tests were taken within the organized screening program and the coverage was 80% for women ages 23-60 (76).

In Sweden, we have seen a dramatic decrease in cervical cancer incidence and mortality since organized screening was introduced in the late 1960s. Following the introduction of screening, there was a significant increase in carcinoma in situ (CIS), a downstaging pattern that would be expected with the implementation of screening. In recent years the cervical cancer incidence has remained stable, with some increase in adenocarcinoma (Figure 3). This raises questions as to whether we have reached the performance plateau of cytology-based screening in Sweden. By switching to HPV-based screening, we could increase the sensitivity of screening. Cytological cervical screening has been shown to be more effective for the

prevention of SCC than adenocarcinoma, perhaps as a result of greater ease in sampling the squamo-columnar junction (77).

Figure 3.



That said, perhaps holding the incidence steady is a sign of continued screening progress given the increasing incidence of precancerous lesions in the population, especially among young women (76). One study has shown that in the absence of screening, the Nordic countries would be experiencing incidence rates on par with high incidence rates in low-income countries (78).

National recommendations are proposed by the National Board of Health and Welfare (Socialstyrelsen) and then implemented at the regional level. Screening is carried out by midwives at maternity care centers. There are some variations in invitation procedures and age-ranges. The intent with the current guidelines was to invite and test every woman up to and including age sixty, but in many regions this was interpreted as no invitations or testing after age sixty. Therefore, many women were not tested after the age of fifty five, and many of the oldest women were never invited. For women entering the screening ages, the first invitation to screening is either sent the year the woman turns 23 which results in some 22 year olds being screened or once the woman turns 23.

The Swedish program is monitored and evaluated at both the national and regional levels. All regions report individual-level screening data to the Swedish National Cervical Screening Registry, described in more detail in the methods section (Nationellt Kvalitetsregister för Cervixcancerprevention, NKCx). The NKCx evaluates the overall effect of the screening program and a process register, Cytburken, evaluates whether screening is

carried out according to the guidelines. Changes to the existing guidelines are made by the National Taskforce for Cervical Cancer Prevention (Nationella Arbetsgruppen för Cervixcancerprevention, NACx), at the request of the national collaboration of Regional Cancer Centers. Initiatives from professional organizations have also helped shape screening in Sweden. The expert group of the Swedish Society of Obstetrics and Gynecology (Svensk Förening för Obstetrik och Gynekologi, SFOG) issued national guidelines for the management of screening test results in 2010. Laboratories are evaluated externally by quality assurance organizations. Regional steering groups in conjunction with region cancer centers are responsible for quality assurance, follow-up, and changes to the system. The steering groups consist of the head of the invitation system, the head of cytology, a maternity care physician or the coordinating midwife, an oncologist, and an STI expert is recommended in some instances.

Cervical cytology is currently used as the primary test (LBC is used in 20 of the 21 regions) but the decision to switch to primary HPV-based screening was made in the fall of 2014 and a recommendation has been drafted and released for commenting by the National Board of Health and Welfare. A final recommendation is expected this summer, 2015. The proposed intervals, tests, and age-ranges are as follows:

Table 2. Proposed screening schedule for primary HPV-based screening Sweden (52)

Age-range	Test	Interval
23-29	Cytology	3 years
30-49	HPV DNA test	3 years
50-64	HPV DNA test	7 years

The proposed screening schedule will not, in its current form, reduce the number of screening tests over a lifetime. Instead, by switching to a more sensitive test in the ages 30-49 and extending the upper-age limit, we can strengthen the screening program's effect and further reduce incidence of invasive cancer. Keeping cytology testing for the first three screening tests among women ages 23-29 reflects a desire to avoid overdiagnosis, given the high prevalence of HPV in this age-group. Using HPV DNA testing as the primary screening test means that cytology will be used as the reflex test. Women who are found to be HPV-positive above the age of 30 will be triaged with cytology and referred to colposcopy if cytological abnormalities are found. Since the first vaccinated cohorts will be entering the screening ages soon, further adjustments may be needed as this current proposal does not officially take into account vaccination status and testing methods and intervals could be adjusted.

2.9 HPV VACCINATION

Two prophylactic vaccines for HPV have been developed and registered with the European Medicines Agency (EMA): Cervarix™, a bivalent vaccine protecting against HPV types 16 and 18 developed by GlaxoSmithKline and Gardasil™, a quadrivalent vaccine protecting against HPV types 6, 11, 16, and 18 developed by Merck. The quadrivalent

vaccine was available on the Swedish market in the fall of 2006 and the bivalent vaccine came a year later. In HPV-negative women, the bivalent and quadrivalent vaccines have been shown to have a 99% and 100% efficacy against HPV 16 and 18 CIN2 or CIN2+, respectively and 65% (bivalent) and 43% (quadrivalent) efficacy against CIN2+ irrespective of HPV type (28).

A 9-valent prophylactic HPV vaccine, developed by Merck, was approved by the U.S. Food and Drug Administration in December 2014 and a positive opinion on recommending it for authorization was released by the EMA in March of 2015 (79). Compared to the quadrivalent vaccine, the antibody responses achieved with the 9-valent vaccine for types 6, 11, 16, and 18 were non-inferior compared to the quadrivalent vaccine. For HPV types 31, 33, 45, 52, and 58, the efficacy against high-grade cervical, vaginal, and vulvar lesions in the per-protocol population of the vaccine trial was 96.7% (80).

Efficacy from the trials provide an estimate of the vaccine impact in an ideal setting; since implementation of vaccination in population, further studies have examined vaccine effectiveness (81). Studies examining vaccine effectiveness against early HPV-disease endpoints have reported decreases in condyloma incidence (82, 83) and a herd protection effect for heterosexual males when females are vaccinated (84). More recently, effectiveness against cervical abnormalities has been shown (85). Reported adverse events following vaccination have been mainly mild in nature, namely swelling and pain at the injection site and fever (86, 87). Population-based safety studies examining the vaccines have shown them to be well-tolerated with no evidence to support an association between quadrivalent HPV vaccination and autoimmune, neurological, and venous thromboembolic events (88) and no evidence of an association between quadrivalent HPV and multiple sclerosis or other demyelinating diseases (89).

The vaccines were originally recommended in 3-dose schedules given at 0, 2, and 6 months for Gardasil and 0, 1, 6 months for Cervarix. Following further immunogenicity studies, non-inferiority of 2-doses compared to 3-doses was demonstrated for young women (90). WHO has reviewed the existing evidence on dosing and they updated their recommendations in 2014, approving a 2-dose schedule for use in young girls (91). Using a 2-dose schedule may reduce costs and logistical challenges of achieving high coverage, but will need to be monitored to ensure similar effect once implemented in population.

Currently, the vaccination is recommended for women ages 9 or older. There is no upper age limit for the quadrivalent and bivalent vaccines as studies have shown efficacy through age 45 (92, 93). Vaccination of males is hotly debated, especially given that males are carriers of HPV and increasing evidence that males are at risk for a variety of HPV-related cancers. Vaccination of both genders is implemented in a limited number of countries: Australia, Austria, Canada and the United States (94, 95).

2.10 HPV VACCINATION IN SWEDEN

The bivalent and quadrivalent vaccines were made available at a subsidized price for girls ages 13-17 in 2007. HPV vaccination was opportunistic, meaning that individuals had to seek and request vaccination at their primary care provider or a vaccination center until 2012

(96). The vaccination coverage achieved during the opportunistic period was approximately 30%, on par with what has been seen with other non-organized HPV vaccination efforts (97). Organized, school-based vaccination began in 2012 targeting girls ages 11-12 with a catch-up of girls ages 13-18. The age-range for vaccination in Sweden was chosen based on early modeling studies using HPV serology data (98).

By 2014, ≥1 dose coverage was 82% for the first cohort of girls in the school-based program (born 1999-2001) and 59% for girls in the catch-up target ages (born 1993-1998) with variations between regions (99). Vaccination up to the age of 26 has been promoted in two regions of Sweden, Stockholm and Skåne. In Stockholm, the vaccine is free for this extended age-group and the coverage in 2014 was approximately 20%. Vaccination of boys is allowed in Sweden, but no organized efforts are in place to systematically invite or encourage vaccination among boys. In accordance with the new recommendations from the WHO, Sweden decided to switch to a 2-dose schedule for the routine school-based vaccination of young girls, effective January 2015 (91, 100).

3 AIMS

Through epidemiological studies of cervical screening and incidence of precancerous cervical lesions and cervical cancer, an investigation of organization and quality assurance in screening and vaccination programs, and mathematical modeling comparing HPV vaccination scenarios, this thesis sought to inform optimization of cervical cancer prevention by means of screening and HPV vaccination.

The specific aims of the studies are as follows:

Paper 1: To obtain estimates of the relative efficacy of HPV-based versus cytology-based screening using pooled data from four randomized trials; to determine how efficacy changes according to age, cancer stage, and morphological features; and to estimate the duration of protection against cancer by screening method.

Paper 2: To assess whether the increased sensitivity of screening with HPV-based testing may represent overdiagnosis and to compare the long-term duration of protective effect against CIN2+ in HPV- and cytology-based screening using data from the long-term follow-up of a randomized controlled trial of primary HPV screening.

Paper 3: To evaluate the HPV type-specific long-term ARs, PARs, and IRRs for low-grade lesions, in the context of a population-based randomized controlled trial. Secondarily, to quantify whether surveillance bias caused by clinical intervention based on a type-restricted HPV test materially affected the risk estimates.

Paper 4: To characterize current organization and quality assurance of screening programs in Europe and to estimate the financial resources required to monitor them using a questionnaire circulated to all EU/EFTA countries.

Paper 5: To describe progress with implementing organized HPV vaccination programs in EU/EFTA countries and to investigate vaccination program monitoring and evaluation strategies and associated program costs.

Paper 6: To compare the impact of different vaccination scenarios on HPV infection control among women and to assess the resilience of each vaccination strategy to a temporary reduction of coverage, using real-life data from the Swedish vaccination program.

4 FUNDING

My thesis work was generously supported by the European Community's Seventh Framework program (FP7/2007-2013) under grant agreement No. 242061 (acronym PREHDICT) and then by the European Commission's Seventh Framework program (FP7/2007-2013) under grant agreement No. 603019 (acronym CoheaHr). It was further supported by the Swedish Foundation for Strategic Research (SSF), under the Advancing Cervical Cancer Eradication Strategies (ACCES) project. Support was also received from the Robert Lundberg Memorial Foundation at the Karolinska Institute, which aims to promote scientific research on tumor diseases.

5 MATERIALS AND METHODS

5.1 CANCER AND INFECTIOUS DISEASE EPIDEMIOLOGY

Epidemiology is "the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to control of health problems" (from J.M. Last as quoted in dos Santos, 1999 (101)). Epidemiologists are interested in what causes disease in a population and therefore they are equally concerned about those that develop disease and those that do not and what the potential differences may be. Using this information, prevention strategies can be developed to target specific risk factors and early markers of disease (50, 101).

Cancer epidemiology, as a field of study, began developing in the mid-20th century as deaths from infectious diseases declined in high-income countries. Noteworthy studies on the link between smoking and lung cancer and bladder cancer incidence in chemical industry workers influenced the development of exploring cancer etiology. Typically, cancer takes years to develop and occurs relatively infrequently in populations, making it logistically challenging to study: large study populations and extended follow-up time are required to observe outcomes. Cancer epidemiology usually includes not only the study of cancer itself, but also its precursors, and as such, it is also interested in the prevention of disease and identifying risk factors for developing disease (101).

Infectious disease epidemiology concerns itself with the spread of infections through contact (direct and indirect), vectors, air, food and water and corresponding disease outcomes in changing populations of susceptible, immune, and recovered individuals. When studying infectious diseases, the likelihood of transmission, the severity of disease caused by the infection, the duration of the infectious period, and the level of immunity gained by having been exposed are some important determinants of estimating the impact of an infection in population (102). In 2008, approximately 2 million or 16.1% of all cancers in the world were attributable to infections, and of those 2 million cancers, 30% could be attributed to HPV (103).

Cervical cancer research is uniquely positioned at the intersection of infectious disease and cancer epidemiology. When studying cervical cancer prevention, the hardest endpoint, and ultimate measure of impact of prevention efforts in population, is estimating reductions in invasive cancer incidence and mortality. Measuring HPV prevalence in population provides an early measure of primary prevention and can give us insight into the likelihood of eliminating HPV. Precursor lesions give us a sense of HPV prevalence in population and can be used to evaluate detection rates in screening and impact of vaccination. Estimating overall burden of disease – precursors and cancer – allows health systems to plan resource allocation and evaluate cost-effectiveness of prevention strategies.

5.2 STUDY DESIGN

A range of study designs have traditionally been used in epidemiological studies of cancer and applied according to the type of research question posed. Study designs are

broadly divided into observational (cohort, case-control, cross-sectional) and experimental studies (randomized controlled trials and quasi-experimental designs). The main difference being that in the former, the researcher observes and reports on what occurs in a population but does not actively make changes and in the later, the researcher intervenes and then observes what happens as a result of the new treatment, screening method, vaccine etc. The data used in this thesis are primarily from randomized controlled trials (RCT), a study design that can be considered to be ideal for evaluating new interventions and their (potential) side effects (50).

In a RCT, consenting study participants are randomly assigned to receive the new intervention or a control intervention and then followed for the outcome of interest. Typically, RCTs are blinded, meaning that information on who receives the new intervention is kept secret during the course of the study so as not to influence the behavior of the participants or the follow-up/care they receive in the study. RCTs can be double blinded, meaning that both those that are implementing the intervention and the participants themselves are unaware of the randomization status. In some instances, trials can even be triple blinded, in which case those analyzing the data receive coded information that does not reveal the randomization status.

RCTs are uniquely positioned to provide information on whether the exposure (intervention) of interest is casually associated with the outcome. The randomization process aims to make the different analysis groups comparable with regard to both observable and non-observable factors aside from the intervention that may influence the outcome. By keeping the groups the same except for the intervention, the effects observed can be attributed to intervention, assuming that other potential sources of bias can be minimized. Results from RCTs are presented as comparisons between analysis groups, often referred to as the intervention and control groups.

Not all interventions are well-suited for randomization. Withholding an intervention we know to be effective can be ethically difficult unless further evaluation of the intervention is justifiable; likewise, we cannot randomize individuals to an intervention or a lack of intervention that we know to be harmful. Ongoing monitoring of study results is critical to ensure that if the benefits or harms exceed expectations, we can adjust the study protocol accordingly or stop the intervention. We must weigh the risks and benefits associated with each intervention we want to evaluate and carefully select the study setting best suited for examining the intervention's effects.

5.3 DATA SOURCES

5.3.1 Overview of data sources and methods by study

	Population or Setting	Material	Method
I	Women attending organized cervical screening in England, Italy, the Netherlands, and Sweden	Long-term follow-up of the European randomized controlled trials on primary HPV screening. Data from the ARTISTIC (England), NTCC (Italy), POBASCAM, (the Netherlands), and Swedescreen (Sweden) trials. Data linkage to NKCx for Swedescreen through July 2011 in Skåne, September 2011 in Gothenburg; January 2012 in Stockholm, and March 2010 for all other regions.	Kaplan Meier curves Cumulative incidence Detection rate ratios
II	Women ages 32-38 attending organized cervical screening in Sweden	Long-term follow-up of the Swedescreen trial. Data linkage to NKCx through September 2011 for Gothenburg, Janaury 2012 for Stockholm, and December 2011 for all other regions. Baseline HPV information from both study arms used. HPV testing performed on frozen baseline samples from the control arm.	Kaplan Meier curves Cumulative incidence Longitudinal test characteristics
III	Women ages 32-38 attending organized cervical screening in Sweden	Long-term follow-up of the Swedescreen trial. Data linkage to NKCx through December 2012 for all regions. Baseline HPV information from both study arms used. HPV testing performed on frozen baseline samples from the control arm.	Absolute risks Incidence rate ratios Population attributable proportions
IV	29 EU/EFTA countries	Survey data collected through the European Union survey on organization and quality control of cervical cancer screening and HPV vaccination programs	Descriptive data on screening program organization and quality assurance
V	27 EU/EFTA countries	Survey data collected through the European Union survey on organization and quality control of cervical cancer screening and HPV vaccination programs	Descriptive data on vaccination program organization and quality
VI	Swedish HPV vaccination program	HPV prevalence (chlamydia screening program) Sexual behavior (Sex in Sweden) Fertility, mortality, and population data (SCB) Vaccination coverage (Folkhälsomyndigheten and personal communications)	Percent reduction of HPV attributable to vaccination Relative cumulative number of vaccine doses

5.3.2 Registers and register linkages

The Swedish national population-based registers enable much of the epidemiological research that is produced in Sweden and allow for unique, longitudinal and population-level studies of health conditions. There are registers that contain demographic information (held by Statistics Sweden) and registers that contain information related to health outcomes, prescriptions, and healthcare visits (held by the National Board of Health and Welfare). In addition, there are quality registers that are used to monitor and evaluate specific health services and outcomes using clinical data, with the overall aim of improving healthcare provision and supporting research and healthcare management.

Registers in Sweden contain individual-level data, identifiable and linkable through a personal identity number (104). Access to registry data varies by type of information included but for registries containing health information, access is strictly regulated by register holders and extracts are only granted after ethical review and proof of data protection measures. The Swedish healthcare system is tax-payer funded and offered in a largely decentralized manner where equal access to care and reasonable timeframes for receiving care are a priority. Health registers allow for administrative review of the quality and effects of healthcare services. This thesis makes use of RCT databases and three registers, as described below.

The Personal Identity Number and data linkages

Personal identity numbers (PIN) were introduced in 1947 and are given to all Swedish citizens and immigrants who become permanent residents or plan to reside in Sweden for more than one year. The number consists of 10 digits: six digits corresponding to the individual's date of birth and then a four-digit identification number (the third digit shows the individual's gender and the last digit is a control number). The PIN is used in all aspects of civil life, vital statistics, and healthcare services to efficiently and accurately identify individuals (104). For research, the PIN enables correct assessment of the population size for calculating national statistics, and individual-level linkages between registers and between primary data collection and registers for research. It allows for the accurate longitudinal follow-up of individuals through changes in residence status, healthcare visits, and health outcomes. The PIN is used to link data from different registers but then is removed before analysis and replaced with a study identification number to protect individual's privacy.

The Swedish National Cervical Screening Registry (Nationellt Kvalitetsregister för Cervixcancerprevention, NKCx)

The Swedish National Cervical Screening Registry (NKCx) was started in 2002 with the aim of building an evidence-base for monitoring and evaluating cervical cancer prevention in Sweden. Data on all cytologies and histologies are included in the database with information dating back to the 1970s for some counties and complete information for all

counties since the early 1990s. Information on invitations to screening is included from the 1990s and onwards (complete information after 2005). Data on HPV tests carried out either as primary screening or triage/test of cure are also collected. Laboratories and regional screening organizations export their data and send copies to the registry. The data are cleaned, standardized, and made available for research and program evaluation. Systematized nomenclature of medical diagnoses (SNOMED) coding according to the Swedish Society of Clinical Cytology is used by the registry to classify cytology and histology results. The codes that are approved for use in Sweden are shown in Table 3. CIN terminology is used for histological test results.

Table 3. Swedish standard cytology nomenclature (Sverigeremissen), 2013

Category	Description	SNOMED
Sample quality	Inadequate	M09010
	Endocervical cells lacking	M09019
Normal	Benign	M00110
Squamous cell diagnosis	ASCUS	M69710
	Mild dysplasia (CIN1/LSIL)	M74006
	ASC-H	M69719
	Moderate dysplasia (CIN2)	M74007
	Severe dysplasia (CIN3)	M80702
	Squamous cell carcinoma	M80703
Glandular epithelial cells	AGUS	M69720
	Adenocarcinoma/AIS	M81403
Uncertain/other cell type	Atypia in cells of uncertain origin	M69700
	Malignant neoplasm of uncertain origin	M80009

The registry is led by a steering group of experts from different disciplines within cervical cancer prevention. Reports are produced each year that provide information and feedback on data completeness and key quality indicators (76).

The Swedish Cancer Register (SCR)

The Swedish Cancer Register was started in 1958 and is held by the National Board of Health and Welfare. It covers the whole population and reporting of malignancies is mandatory; reports from all examinations (clinical-, morphological -, and other laboratory examinations) must be sent. The six regional cancer registries, within the regional cancer centers, are responsible for the initial cleaning and checking of the data submitted. Data are then compiled at the national level in the Swedish Cancer Registry, containing detailed information on the cancer, its diagnosis, and the reporting entity. Information on staging has been systematically included since 2004. The completeness of the registry was evaluated in 1998 by comparing cancer cases reported to the cancer registry with those reported to the Hospital Discharge Registry. Underreporting of cancers was found to be age-dependent with

more underreporting in older ages for women. The underreporting of female genital cancers was found to be low (3.4%) (105).

The Swedish Total Population Register (TPR)

Records of the Swedish population have been kept since the 1600s, when church parishes started to keep track of their members. The system was computerized in the 1960s and later transferred to the tax authority in 1991. Demographic information including births, deaths, civil status, place of residence, immigration, and emigration are reported by local tax authorities and compiled by the national tax authority. Such information is then reported to the Total Population Register, complete since 1968 and held by Statistics Sweden (Statistiska Centralbyrån, SCB) (106).

Swedescreen

Data for the first three studies of this thesis come from the Swedecreen trial, which was started in 1997 as the Swedish randomized controlled trial of primary HPV-based screening. A total of 12,527 consenting women, aged 32-38, attending population-based invitational screening in Sweden were randomized 1:1 to HPV test and cytology (intervention arm) or cytology only with samples frozen for future HPV DNA analysis (control arm). The randomization was performed independently by the Cancer Registry of Stockholm using computer-generated numbers. Women were recruited between May 1997 and November 2000 in Göteborg, Malmö, Stockholm, Umeå, and Uppsala. Inclusion criteria were simple, women needed to consent to participate in the study. No exclusions were made based on previous screening status or history. HPV-positive women were invited for a second HPV test at least one year later and women with type-specific persistent infections were then invited to colposcopy. A similar number of random double-blinded procedures were performed in the control arm to address possible ascertainment bias (107). Women are followed with comprehensive registry-based follow-up. The primary outcome was the relative rates of CIN grade 2 or worse (CIN2/CIN3+) found in subsequent screening. Secondary outcomes were the relative rates of CIN2/CIN3+ found in baseline screening and outcomes stratified by grade of CIN (CIN2 or CIN3+). The study was unblinded in August 2003 and women were informed of their HPV results because the proportion of women who were HPV positive and found to have a CIN2+ lesion was greater than expected (108).

The Swedescreen study data contain all the original information collected at baseline for the study participants and the interventions conducted, including follow-up HPV test results and results of the study colposcopies. A long-term follow-up of the cohort has been completed with individual linkage to NKCx. This long-term follow-up includes all cytology and histology test results for the cohort. Raw cytology and histology diagnoses were cleaned using the Swedescreen SNOMED code translation table, which was updated and expanded for the studies in this thesis. In 2012, HPV testing of the frozen baseline samples in the control arm of the trial was completed. This information is available in the long-term follow-

up data. Additional censoring information (death or migration status) was collected from Statistics Sweden and used in Study I. Information on cancer cases during follow-up is also included.

Other primary HPV-screening RCTs in Europe

Closely following the Swedescreen trial, primary HPV screening trials were started in other European countries to compare the effectiveness of HPV- to cytology-based screening with precursor lesions as an endpoint: the ARTISTIC (A Randomized Trial In Screening To Improve Cytology) trial in England (2001), the New Technologies for Cervical Cancer screening (NTCC) trial in Italy (2002), and the Population-Based Screening Study Amsterdam (POBASCAM) trial in the Netherlands (1999). All studies recruited from routine screening within organized, population-based screening programs. The age-ranges differed somewhat, reflecting country-specific screening differences and a desire to examine HPV-based screening in different age groups: ages 20-64 in ARTISTIC, 25-60 in NTCC, and 29-61 in POBASCAM. In NTCC and POBASCAM women were randomized 1:1 as in Swedescreen to HPV (intervention) and cytology (control arm) testing, whereas in ARTISTIC, women were randomized 3:1. Individual, study-specific analyses have been published explaining the studies and baseline data, risk for precursor lesions, and, more recently, longer-term follow-up of study results (66, 108-113).

5.4 STATISTICAL METHODS

5.4.1 Study I

As the European primary HPV screening trials were not separately powered to look at cancer as an outcome, in order to be able to study the screening test effect on invasive cervical cancer, data from the ARTISTIC, NTCC, POBASCAM, and Swedescreen were pooled. This study was a joint effort within the EU FP7 project, PREHDICT. Data were collected and in each country and prepared for pooling according preset definitions. For Sweden, cases were identified through linkages to the pathology data in NKCx and the SCR. The diagnostic slides for these cases were collected from biobanks and were then reviewed by an expert pathologist (WR). Of the 23 potential cases identified, 20 were reviewed by the pathologist (the remaining 3 were found only in pathology registry or only in cancer registry and considered not confirmed). Twelve cases were confirmed as invasive cervical carcinoma at the specimen's blind re-review and included as cases in the pooled analysis. Cases were further classified by morphological features (squamous cell carcinoma, adenocarcinoma, and adenosquamous) and FIGO stage (1A vs >1A). Baseline information on HPV status was obtained from the original data and follow-up tests were obtained through linkage to the NKCx for all cytological and histological results (follow-up through July 2011 in Skåne, September 2011 in Göteborg, January 2012 in Stockholm, and March 2010 for all other regions).

The data were analyzed by intention to screen – analyses were conducted according to study arm and person-time counted from recruitment until the end of follow-up, cancer detection, death, or migration, whichever came first. Invasive cervical cancer is most often symptomatic meaning that diagnosis is not necessarily screening-dependent. Person time was counted until the end of the registry linkage when no case or migration/death was observed. This is dissimilar to how follow-up time was counted in Studies II and III where precursor lesions, which are most often asymptomatic and are detected through screening, were used as the outcome and last date of screening was used as the end of follow-up for individuals who did not experience a lesion.

Cumulative incidence of cervical cancer by study arm and then by baseline test result status (HPV negative in the intervention arm and cytology negative in the control arm) was calculated using the Kaplan-Meier (KM) method. All studies except ARTISTIC used a 1:1 randomization to study arms. Since ARTISTIC used a 3:1 randomization, the results of the crude KM could be biased. An adjustment was made by multiplying the intervention arm women at risk and cases by 0.5 and the control arm by 1.5. Study-adjusted detection rate ratios were calculated for invasive cervical cancer in the intervention versus the control arm. Rate ratios were calculated for the overall observation period, and then for the first 2.5 years of follow-up accounting for the prevalence screen and the period thereafter, separately. Further analyses included calculating rate ratios for women with a negative test at entry, and by morphology, stage, age at enrolment, and proportion of women with at least 1 biopsy result to explore the extent of diagnostic procedures.

Given that the study design and implementation were somewhat different across the trials included, heterogeneity was assessed. Heterogeneity arises when there is between-study variation in the study results that is greater than what could be expected by chance (114). For this analysis, heterogeneity was assessed with a χ^2 test and the I^2 statistic. The χ^2 provides a statistical test of homogeneity which is specific but not always sensitive, meaning that large p-values do not necessarily mean that heterogeneity can be ignored (115). Therefore, the I^2 statistic can be useful as it shows the proportion of the variance in the effect estimates that is due to heterogeneity between the studies included in the pooled analysis (0% means variability is due to sampling errors in the studies and not heterogeneity between studies and 100% means the variability is due entirely to heterogeneity between the studies) (114). The main results of the analysis were generated from fixed-effects models which assume that each of the studies estimate similar exposure effects but as a further check, random effects models were run as well where the exposure effects were allowed to vary between studies (115).

5.4.2 Study II

This long-term follow-up of the Swedescreen trial made use of both study arms and the updated HPV testing in the control arm. Baseline test results were categorized as HR HPV positive, HR HPV negative, cytology negative (normal), and cytology positive (ASCUS or worse). Analyses were completed by baseline test result and study arm. Women with unsatisfactory baseline cytology or missing baseline cytology were excluded when examining cytologies; women with unknown HPV baseline result were excluded for the HPV analyses;

and women with both unknown HPV and an unknown cytology result were excluded in the cytology and HPV analyses. Follow-up began at the first study test result (HPV or cytology at baseline) and ended at the first histologically confirmed CIN2+ or CIN3+ lesion, the last registered sampling date, or 13 years of follow-up, whichever came first. The last registered sampling date was used as the end of follow-up for individuals who did not experience an outcome since high-grade lesions are typically screen-detected and we wanted to include only observation time when outcome status was known. Information on cytologies and histologies was obtained through linkage to the NKCx with follow-up through September 2011 for Göteborg, January 2012 for Stockholm, and December 2011 for all other regions.

Cumulative incidence by study arm and baseline test result were calculated as 1 minus the Kaplan Meier curve (the complement of the negative predictive value, cNPV). Longitudinal test characteristics, sensitivity specificity, positive predictive value (PPV), and negative predictive value (NPV), were calculated adjusting for censoring as a measure of how well a baseline test result can predict future occurrence of disease. Typically, test characteristics are calculated and compared to a gold standard at the same point in time. In this case, we measured the gold standard (a histologically confirmed high-grade lesion) during follow-up which complicates matters as the women included at baseline were not necessarily followed for the entire period of interest. Therefore, the numerator and denominator must be upweighted as if the censored women were still at risk using the censoring distribution estimated from the Kaplan Meier curve. Conditional weighting was used to reflect that censoring may depend on the baseline test result (116). We calculated test characteristics at 3, 5, 8, and 10 years of follow-up to reflect existing and proposed screening intervals. A two sample test of proportions was used to assess sensitivity of cytology in the control arm at 3 years (the recommended interval) compared to sensitivity of HPV testing in the intervention arm at 3, 5, 8, and 10 years. Overdiagnosis was assessed visually and by overall counts of outcomes in the control and intervention arms. The concern has been that HPV testing results in overdiagnosis; therefore, for there to be no evidence of overdiagnosis, the number of cases in the control arm should catch-up with the number in the intervention arm over long-term follow-up (60).

5.4.3 Study III

Data from both arms of the Swedescreen trial were used in this analysis along with the updated HPV testing in the control arm. The updates to the testing method used in the control arm allowed for typing of non-HR HPV types as well. Individuals were followed from their first study test results (HPV or cytology) until the first study diagnosis of an ASCUS, LSIL (cytology), or CIN1 (histology) diagnosis depending on the analysis or the last registered cytology, if no outcome was observed. Women who were diagnosed with a CIN2+ lesion before a low-grade lesion were censored with the assumption that treatment for the CIN2+ lesion would have changed the course of their disease history. Information on disease outcomes and screening episodes were obtained through a linkage to the NKCx, with a country-wide linkage through December 2012.

To explore the HPV type-specific risk for low-grade lesions, a series of estimates were calculated. Type-specific cumulative incidences (absolute risks, AR) were calculated at 14-years of following using 1-Kaplan Meier curve to account for censoring given the extended follow-up time. Absolute risks give a measure of the risk that a woman testing positive for a specific HPV type will develop the disease of interest in the time specified. This measure is useful for making clinical predictions but, unadjusted ARs do not provide evidence for whether that infection caused the lesion.

Unadjusted and adjusted incidence rate ratios (IRR) were calculated to provide an estimate of how much the risk of low-grade lesions is increased among women positive for a specific HPV type compared to women negative for that time in a particular timeframe and how much this increase is actually caused by the type-specific infection after adjusting for co-infections. The IRRs were calculated using Poisson regression and adjustment for co-infections was handled by entering individual type information into the model. Follow-up time was used as the timescale and entered as a covariate in the models. We measured follow-up time as time-since-entry into the study; this was chosen since HPV status was measured at entry into the study and follow-up time gave a measurement of time since HPV status was known. A Wald test was used to test whether the IRR for each type was different than that of the IRR for type 16.

The population attributable proportion (PAR) was calculated to estimate the proportion of disease that would be eliminated if the infection was prevented in the population. This measure is of particular interest now that we have highly efficacious vaccines that can prevent infection with specific types. Estimating the PAR gives us a sense of what we can expect from the vaccine in terms of impact on low-grade lesions. Type-specific PARs were calculated for the first screening round in the study as the adjusted IRR minus 1, divided by the IRR and multiplied by the proportion exposed in the population (117, 118). The estimates were run separately for each study arm and then combined, adjusting for study arm in the model.

5.4.4 Study VI

Mathematical models are equational representations of complex occurrences that help us to study systems. In healthcare research, models allow us to make inferences about the future based on available data and give us an opportunity to simulate potential interventions and their impact on health outcomes. Infectious disease modeling seeks to represent the dynamics of an infectious agent as well as the spread of it in the population (the transmission between individuals). It can be further extended to investigate control measures, such as vaccination (102). Substantial modeling work is currently being done on HPV and HPV-related diseases since the advent of HPV vaccination and a desire to further evaluate prevention strategies. Models differ quite significantly, not only in the modeling approach but also with regard to how the input data are defined and which assumptions are made. Methodological choices in modeling are extensive and require a deep knowledge of the infectious agent in question and its sequelae. The ability of a model to represent reality is often influenced by the quality and detail of existing data sources and assumptions about

uncertainties in the natural history and spread of the disease. The elegance of modeling is finding a parsimonious but valid representation of the system's complexity.

The model used in this thesis was developed by Dr. Iacopo Baussano at the International Agency for Research on Cancer building on work completed in transmission modeling at Imperial College London in the group of Dr. Geoffrey Garnett. Further development is ongoing with the model, but to date, it has been used to explore transmission, clearance, and persistence of HPV as well as upscaling or extending HPV programs in other settings (15, 119, 120). Model validation against Swedish and Italian data has been performed (121). The model is a population-based age-structured transmission dynamic model, accounting for men and women that are susceptible, infected, and immune to HPV infection. Age-structuring the model allows parameter estimates and rates within the model to vary by age. Movement between states (susceptible, infected, and immune) in a dynamic model is based on rates.

For Study VI, we chose HPV prevalence as the outcome of interest because changes in prevalence would be the earliest impact of the vaccination program. Input data were Sweden-specific and were collected and prepared for modeling calculations. HPV prevalence data were obtained from the chlamydia screening program in Southern Sweden (Skåne) (122). Information on sexual behavior was taken from the Sex in Sweden survey, conducted in the late 1990s and one of the most comprehensive data sources on sexual behavior of both men and women (123). The raw data from Sex in Sweden was kindly provided by Professor Bo Lewin at Uppsala University. The average number of new partners in the past 12 months was extracted and the proportion of the population in low, middle, and high sexual activity classes was estimated. We assumed vaccine efficacy according to data from the vaccine trials (28).

The aim of the study was to compare the impact of alternative HPV vaccination strategies and assess vaccination program resilience to a reduction in coverage. Using vaccination coverage data from the Swedish program, 3 alternative scenarios based on a 2-dose vaccination schedule were designed to represent possible expansions of the program and are described in more detail in the main findings section. Resilience of the vaccination program was explored by modeling a 5-year, 50% decrease in vaccination coverage and then comparing vaccination scenarios. Drops in vaccination coverage can be caused by changes in healthcare priorities or political will behind a vaccination program, public mistrust in a vaccine, etc. and have lasting effects on disease outcomes. Mitigating the negative effects of a decrease in coverage is of interest to ensure robust health programs that can withstand temporary changes.

For each scenario proposed, we explored the percent relative reduction in prevalence of vaccine targeted HPV types (RAV) among women ages 15-35 and then by birth cohort. Vaccine types 16 and 18, targeted by the bivalent and quadrivalent vaccines were evaluated in the main analysis and vaccine types 16, 18, 31, 33, 45, 52 and 58 (9-valent vaccine) were included in supplementary analyses. We further calculated the absolute gain in prevalence reduction, calculated as the % RAV of the 3 alternative scenarios as compared to the base case (the current vaccination program). Resilience over time of the vaccination program was calculated as the % RAV estimated with and without the temporary coverage reduction.

Finally, we estimate the cumulative number of doses in each alternative vaccination strategy and compared that to the base-case to provide a comparison of health resources needed under potential expansions of the program.

5.5 SURVEY METHODS

5.5.1 Studies IV and V

While screening exists to a degree in European countries, the organization of screening efforts and the extent to which screening is monitored and evaluated differs. The European Centers for Disease Prevention and Control guidelines for HPV vaccination have encouraged monitoring of vaccination programs. Vaccination efforts differ across Europe as well with regard to level of organization and monitoring and evaluation capabilities. In Studies IV and V, we sought to map the current organization and quality of screening and vaccination programs.

The questionnaire tool was designed after a review of existing EU guidelines, country-specific guidelines and protocols, and published literature on quality assurance and organization. A draft of the survey was circulated among program experts in England, Finland, Norway, and Sweden for feedback on the readability and content of the survey. The questionnaire was also sent to the Screening Quality Assurance Group at IARC for commenting. Feedback was received from England, Norway, Sweden and IARC and incorporated. The full questionnaire has been included in as an Appendix but briefly, the survey included seven main sections, of which four addressed screening and the remaining 3 collected information on vaccination.

The questionnaire was sent, in Word doc form, to ministries of health, key screening program administrators and/or researchers associated with the programs in all 34 EU (including separate surveys sent to England, Northern Ireland, Scotland, and Wales) and European Free Trade Agreement (EFTA) countries. If no response was received, a follow-up email was sent and then new contacts were found. Given the length of the questionnaire and the level of detail requested, we encouraged countries to divide up their responses between departments if needed or work as a team to fill in the questionnaire, sending updated versions when they could. Countries were asked to submit supporting information where possible – standard operating procedures, reports, and guidelines.

Data collection started in May 2012 and continued until March 2014, when the last response was received. Collecting information proved to be challenging as finding individuals willing and able to respond, especially in countries were programs were less defined, was difficult. The filled-in questionnaires and corresponding documents sent by countries were collected into country-specific folders and entered into a Masterfile. The level of detail submitted by countries and consistency in answering questions varied significantly. For publication, the results were analyzed and compiled separately for screening and vaccination programs. Further reflections on the content and process are included in the methodological considerations section.

6 ETHICAL CONSIDERATIONS

Studies I, II, and III are all based on the long-term follow-up of the Swedescreen trial and data from ARTISTIC, NTCC, and POBASCAM were used in Study I. When the Swedescreen trial was started in 1997, the women received information on the trial, the study tests, and follow-up procedures as part of the oral and written consent process. The information highlighted that women could withdraw at any point and offered contact information for questions. The study was granted approval in 1996 by the ethical review board. We requested a new ethical approval for the long-term follow-up of Swedescreen including a new registry linkage, identification of cancer cases, data sharing, and testing of baseline samples in the control arm. As no further study tests were carried out in the long-term follow-up, there was no risk for physical pain or discomfort. We reasoned that conducting HPV testing of samples in the control arm 15 years after the samples were taken made re-contacting the women logistically difficult and less relevant since HPV infections typically clear on their own. Furthermore, all women consented to HPV testing and continued to be invited to routine screening following their inclusion in the study, minimizing the risk for missing a diagnosis. Ethical approval was obtained for the other European trials and all women provided informed consent.

The content of the data collected in **Studies IV and V** should be a matter of public record in participating countries as the information collected had to do with healthcare systems and funding sources. We applied for ethical approval for the questionnaire and received an advisory opinion (rådgivande yttrande). The advisory opinion stated that the study did not fall under the auspices of an ethical review, in part because the data were not personal. In writing the ethical approval, the main issue of concern we weighed was that of the political implications for individuals reporting controversial data for their country (e.g. programs were not operating in-line with guidelines). We chose to present results at the country level, mentioning only generally the affiliation of the responder(s) in an effort to protect the individual responder but also allow for opening a dialogue around how programs compare and can be improved. Since public health practice dictates that it is ethically questionable to engage individuals in prevention programs that do not achieve their goals, the act of sharing information on program performance, while perhaps detrimental to the image of the country program, is an ethical obligation.

No new data were collected for the mathematical modelling in **Study VI**. That said, ethical approval was still applied for and received. Data on sensitive issues is needed for transmission models of STIs – namely information on sexual behavior and HPV infection status. Information on sexual behavior from the Sex in Sweden survey was used in aggregate form and anonymized (no record exists linking those contacted with the survey responses). Similarly, only information on age and gender was known for the data used from the anonymous chlamydia screening program in Skåne, Sweden for HPV prevalence. Population level information was obtained from SCB and the National Board of Health and Welfare. The risk for individuals was minimal as all information was aggregated and then simulated and presented at a population level.

7 MAIN FINDINGS AND DISCUSSION

7.1 STUDY I

A total of 176,464 women were included, contributing 1,214,415 person-years over follow-up. The median follow-up time was 6.5 years (covering 2 screening rounds) and 107 invasive cervical cancers were detected. The overall rate ratio (RR) for cervical cancer in the intervention arm compared to the control arm, was 0.60 (95% CI: 0.40-0.89) for all randomized women with no evidence of heterogeneity between studies (p=0.52 and I^2 0.0%). In the first 2.5 years of follow-up (prevalence screen) cancer detection did not differ significantly between the study arms (RR 0.79 (95% CI: 0.46-1.36) but was significantly lower in the intervention arm thereafter (RR 0.45 (95% CI: 0.25-0.81). Among women with a negative test at baseline, the rate ratios became more pronounced. Comparing HPV negative women in intervention arm to cytology negative women in the control arm, the rate ratio for cancer was 0.30 (95% CI: 0.15-0.60), again with no heterogeneity between studies. As further confirmation, the random effects model showed similar results (RR 0.34 (95% CI: 0.14-0.86).

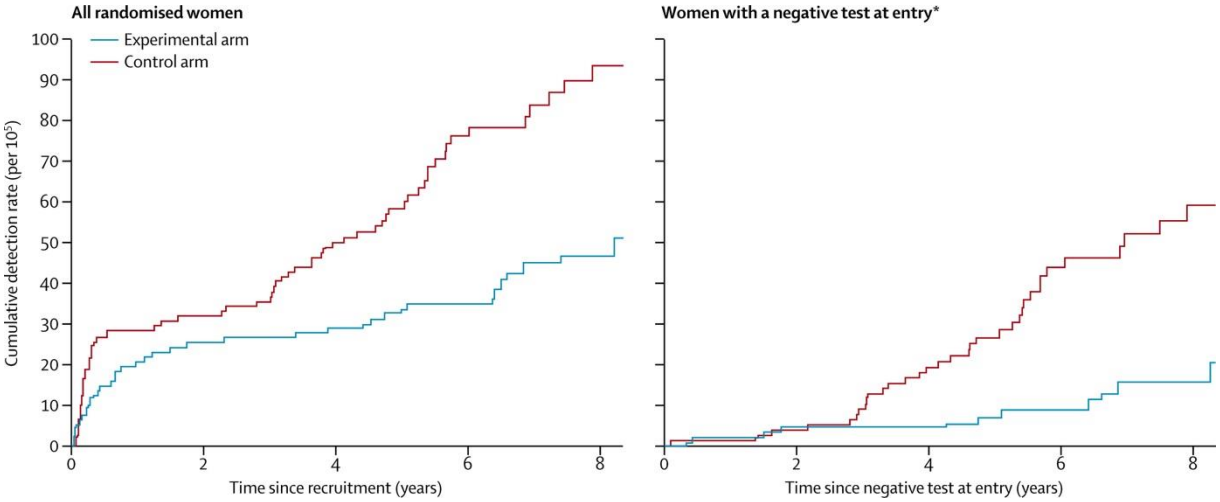
In addition to the main results, we examined several sub-questions of interest. When looking at morphology, the effect for the overall study period was greater for adenocarcinoma than squamous cell carcinoma (RR 0.31 (95% CI: 0.14-0.69) and 0.78 (95% CI: 0.49-1.25), respectively). By age, the effects were greatest for women ages 30-34 (RR 0.36 (95% CI: 0.14-0.94) and no reduction was seen for women below the age of 30. This suggests that HPV-based screening should be implemented for women ages 30 and above.

Previous studies have suggested that HPV-based screening would allow for longer screening intervals that cytology-based screening. The cumulative detection rate (cumulative incidence) for invasive cervical cancer was 15.4 (7.9-27.0) per 10^5 and 36.0 (23.2-53.5) per 10^5 for cytology negative women at 3.5 and 5.5 years after entry, respectively whereas for HPV-negative women, it was 4.6 (1.1-12.1) per 10^5 and 8.7 (3.3-18.6) per 10^5, respectively (Figure 4). The cumulative detection rate among HPV-negative women at 5.5 years was lower than for cytology negative women at 3.5 years implying that lengthened intervals with HPV-based screening would be possible.

To our knowledge, this is the first analysis to focus on comparing efficacy of screening tests against invasive cervical cancer in regularly screened women and to address issues of age, cancer stage, and morphology. A cluster randomized trial in India compared once-in-a-lifetome screening with HPV to cytology, visual inspection, or no screening and found that one-time HPV screening resulted in a reduction of advanced cancers and death (124). In general, results of RCTs of cytology- versus HPV-based screening have been focused on precursor lesions and have had more limited follow-up (125, 126). A previous meta-analysis did not report cumulative incidence since recruitment which means that the observed decrease with HPV-based screening could have been due to earlier detection (127). Our results show that HPV-

based screening provides greater protection against cancer than cytology and that HPV-based screening should be implemented from age 30, extending intervals up to 5 years.

Figure 4. Cumulative incidence of invasive cervical carcinoma



*Observations are censored 2.5 years after CIN2 or CIN3 detection, if any

7.2 STUDY II

Of the 12,527 women recruited to Swedescreen, 12,091 had baseline cytology and at least one follow-up test. Over 13 years of follow-up, 387 women developed a histologically confirmed CIN2+ lesion (median follow-up time for the whole cohort was 10.95 years) and 230 women developed a CIN3+ lesion (median follow-up time for the whole cohort was 10.98 years). The cumulative incidence of CIN2+ among women negative for cytology at baseline in the control arm increased steadily over follow-up whereas the cumulative incidence increased slowly for HPV negative women with very little difference between HPV negative and double negative (cytology and HPV negative) women during follow-up (Figure 5).

Patterns were similar for CIN3+; however, slightly more pronounced differences were seen between HPV-negative and double negative women after 7 years of follow-up, probably due to improvements in the testing method used in the control arm (Figure 6). At 11 years of follow-up, differences in the cumulative incidence of CIN2+ among cytology negative women at baseline in the intervention and control arms diminished, highlighting the earlier diagnosis potential with HPV-based testing and the apparent absence of overdiagnosis when viewing the data over the long-term. Again, similar patterns were observed when comparing cytology negative women in the intervention and control arms with the outcome of CIN3+. The cumulative incidences were somewhat different (higher in the intervention arm) until 7 years of follow-up.

Figure 5. Cumulative incidence of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) over 13 years of follow-up by study arm and baseline test result (test result groups not mutually exclusive)

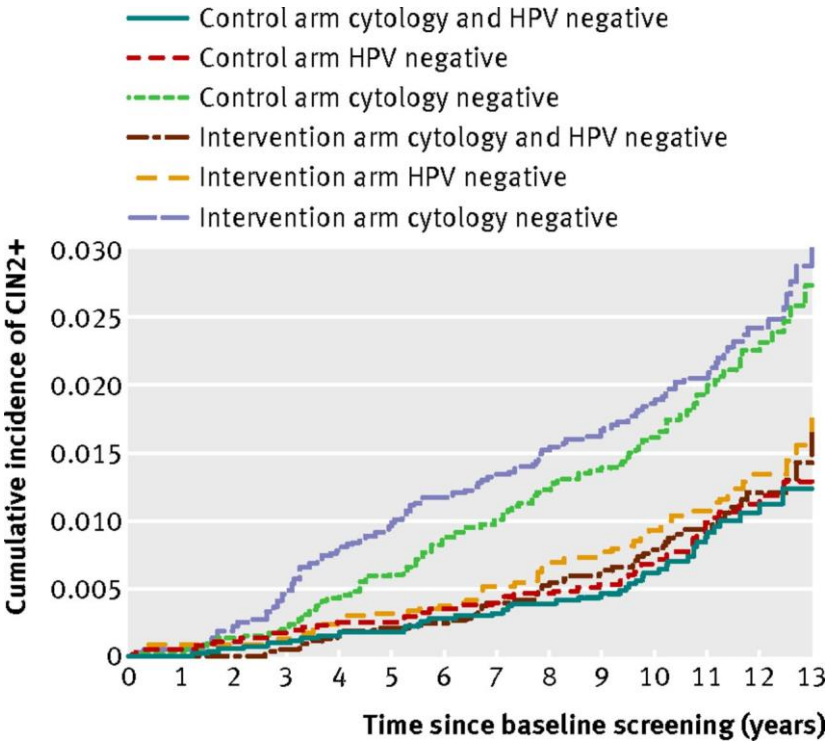
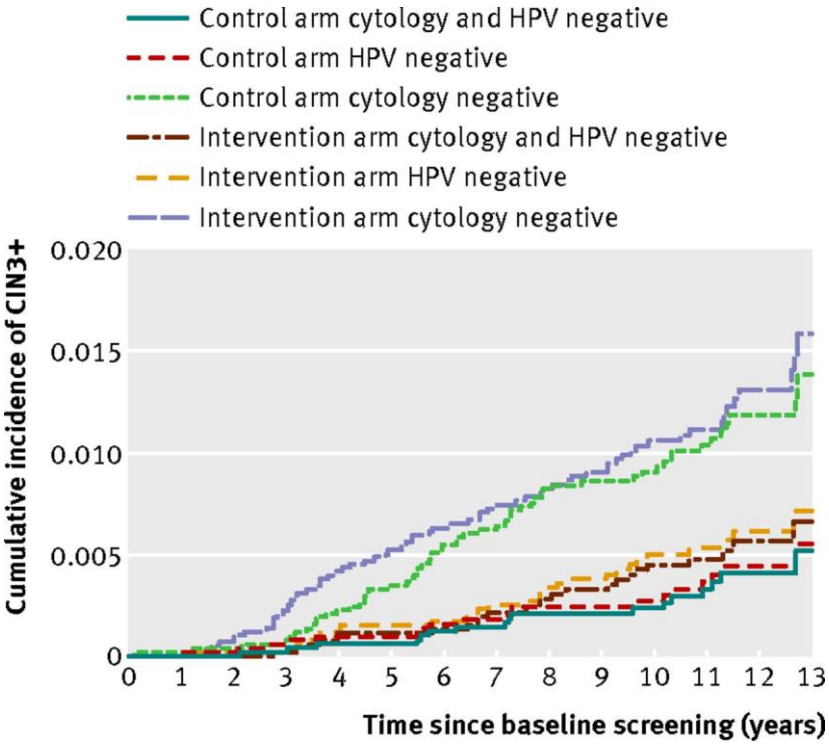


Figure 6. Cumulative incidence of cervical intraepithelial neoplasia grade 3 or worse (CIN3+) over 13 years of follow-up by study arm and baseline test result (test result groups not mutually exclusive)



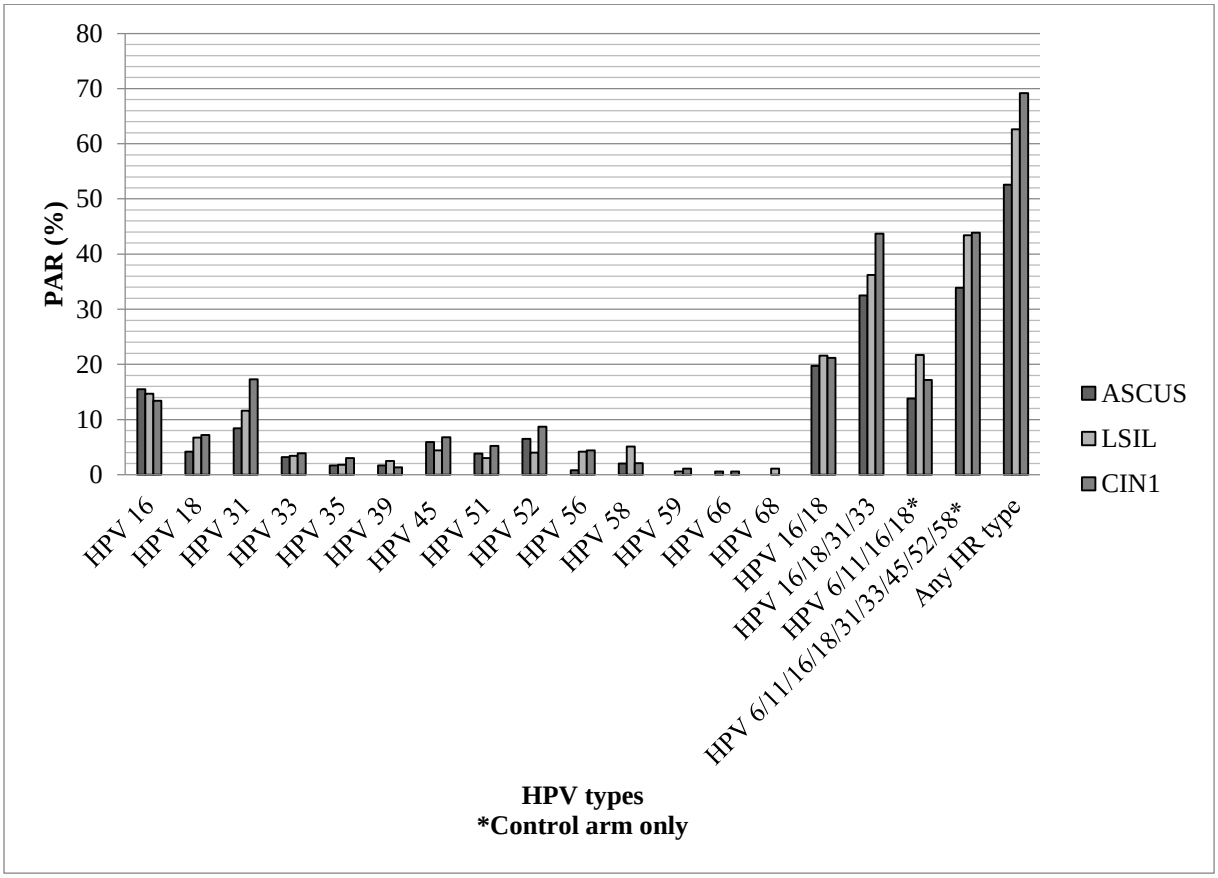
An examination of the longitudinal test characteristics revealed that the sensitivity of cytology in the control arm at 3 years of follow-up was similar to that of HPV testing in the intervention arm at 5 years (85.94% (95% CI: 76.85-91.84) and 86.40% (95% CI: 79.21-91.37), respectively) with no statistically significant difference in proportions (p=0.8970). For CIN3+ the pattern of results remained the same, the sensitivity in control arm with cytology at 3 years was 92.02% (95% CI: 80.59-96.97) and in the intervention arm with HPV testing at 5 years it was 89.34% (95% CI: 80.10-94.58) with no significant difference in the proportions (p=0.4871). As expected with HPV-based testing, the specificity was lower than cytology at all time points but still above 94% for HPV testing and 90% for double testing when looking at CIN2+ as an outcome and above 90% for HPV testing and 85% for double testing with CIN3+ as an outcome.

The drop in specificity for double testing and the minimal gain in sensitivity, suggests that double testing is not likely to offer a pragrammatically worthwhile improvement. Taken together, the results suggest that HPV testing could be used as a stand-alone test and that intervals could be safely extended compared to cytology. The increased detection with HPV based screening the first years of follow-up that has been observed in the first results of Swedescreen as well as other published trials (108, 126, 128), seems to decrease overtime and represent, instead, early detection.

7.3 STUDY III

Study III made use of the same long-term follow-up data from the Swedescreen trial as used in Studies I and II but the follow-up was further extended to December 2012 for all counties. In total, 11,683 women had complete baseline testing (HPV and cytology information) and at least 1 follow-up test were included. The median follow-up time was 11.07 during which 648 ASCUS, 334 LSIL, and 183 CIN1 cases were identified. Analyses were run with study arms separately and then combined and, in addition to the HR HPV types examined in the full cohort, non-HR HPV types were examined in the control arm where updated HPV testing was available. In the control arm, the absolute risk for ASCUS/LSIL was highest for non-HR HPV types 73, 53, 6, and 67. The first screening round PAR for ASCUS, LSIL, and CIN1 separately by type is given in Figure 7 and can be helpful in understanding the proportion of disease that would be eliminated if the infection was prevented in the population. This is of particular interest as it gives us a sense of what we can expect from vaccines. PARs for ASCUS/LSIL were estimated for the bivalent, quadrivalent and 9-valent vaccine types. Given that low-grade lesions are increasing in incidence, these results bode well for reducing the overall burden of low-grade lesions through vaccination.

Figure 7. Population attributable proportion (PAR, %) by type for ASCUS, LSIL and CIN1 in the first 3 years of follow-up, study arms combined



Overall, the IRRs for ASCUS/LSIL were highly follow-up time dependent with the IRR for infection with any HR type decreasing from 18.6 (95% CI: 14.9-23.4) in the first screening round during follow-up to 1.1 (95% CI: 0.7-1.8) in the fourth screening round. Similar decreases were seen when looking at the type-specific adjusted IRRs. It should be noted that we chose to adjust for multiple-type infections by entering all HR types separately into the model. We chose this approach to minimize assumptions and reduce bias. There is discussion in the field on how best to do this – some studies have taken a hierarchical approach to adjust for multiple-type infections while others have done it proportionally (129, 130). Both approaches can bias the results by overestimating the contribution of common oncogenic types (hierarchical approach) or overestimating the contribution of lesser oncogenic types (proportional approach). Given the biases apparent in these approaches and conflicting evidence in the field regarding interactions between types, we chose to adjust for multiple type infections by treating them as confounders.

The results of Study III suggest that type-specific risks for ASCUS/LSIL differ and that most lesions are found during the first screening round. The differences in HPV testing between the intervention and control arms resulted in only a small increase in the proportion of low-grade lesions caused by the types screened for, allowing us to pool the study arms. By

calculating both absolute as well as relative estimates of type-specific risk we hope that the results can be used to assist in healthcare planning by estimating patient burden and evaluate the magnitude of effect of specific types on development of ASCUS/LSIL.

7.4 STUDIES IV AND V

Out of 34 EU/EFTA countries that were sent a questionnaire, 29 countries responded to the sections related to cervical screening and 27 countries responded regarding vaccination efforts. Responses were received from professional organizations, researchers, registries, screening programs, departments of pathology, public health departments, ministries of health, and cancer societies, reflecting the range of actors involved with prevention activities. Data collection took 2 years as some countries required repeated contact before a response was elicited and responses varied in detail and supporting documentation.

Details of program organization and systems for monitoring and evaluation are described in the tables of the manuscripts and provide an overview of the key data points gathered. Briefly, cervical screening was offered to women through an organized program in 20 countries and through a publicly mandated program in 21 countries. Quality assurance programs for screening were established in all but three countries and mass screening registries were in place in all but four countries with individual level data systematically collected in all but three countries. In reviewing the information submitted, it became apparent that each country has approached prevention activities from their own healthcare perspective. While some programs had similar characteristics such screening intervals and target populations, in general programs were integrated into existing systems and implemented according to healthcare tradition in that country. Therefore, the ability to separate costs was more challenging than anticipated. More established programs were able to more fully track screening program operations and provide more detailed responses to the questionnaire items.

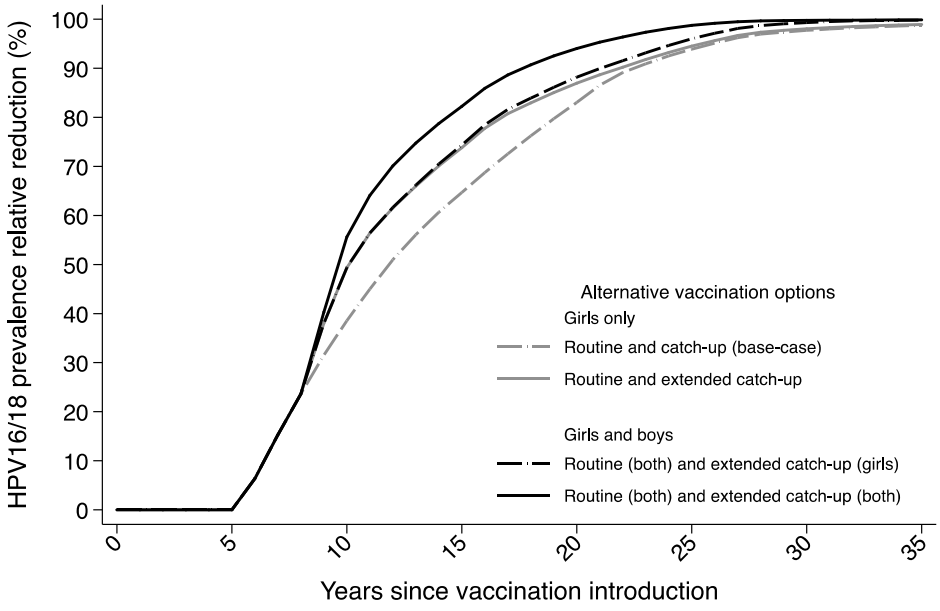
Guidelines regarding how HPV vaccination programs should be implemented are less well-developed and authoritative compared to screening recommendations. The majority of countries surveyed reported having an organized vaccination program with a centralized vaccination registry allowing for monitoring of key performance and impact indicators. Registration of vaccination is ideally coupled with the ability to link information to health outcomes to monitor the impact of vaccination in population and optimize prevention efforts. Six countries reported that they could perform data registry linkages. Among countries reporting an organized vaccination program, 46% reported using a school-based delivery strategy alone and 27% used a school-based strategy along with a secondary delivery strategy. Evidence from a recent meta-analysis suggests that programs that vaccinate girls at a young age and achieve a high-coverage, often through school-based delivery of the vaccine, achieve the greatest impact on HPV-related disease outcomes (131, 132). Continued monitoring and evaluation of vaccine impact in population will be needed with a comprehensive approach to ensuring that the program functions as it should and has the intended effect.

7.5 STUDY VI

Three alternative vaccination scenarios were compared to the base-case (Strategy 1, the vaccination program as it currently is with routine vaccination of school-age girls and a catch-up of 13-18 year old girls). The alternative scenarios included an extended one-time catch-up with high coverage up to age 26 among girls (Strategy 2), adding routine vaccination of school-age boys to the extended catch-up of girls (Strategy 3), and then adding an extended one-time catch-up of boys up to 26 as well, in addition to routine vaccination of both genders and an extended one-time catch-up of girls (Strategy 4). This last option represented the most extensive potential expansion to the program. The outcome of interest in all the analyses was HPV 16/18 prevalence among women ages 15-35 in the years following vaccination program start. By restricting to this age-range, we sought to capture the impact of vaccination in the age-range where HPV prevalence peaks and minimize the effect of different screening practices on the findings.

When looking at the reduction of HPV prevalence attributable to vaccination (%RAV), the strategies including the extended catch-up of females accelerated the reduction in prevalence compared to the base case and increased the effectiveness. Including an extended catch-up of males further sped up the impact with the effects lasting for 25-30 years post-vaccination program start (Figure 8). Overall, results were similar when evaluating RAV for the HPV types included in the 9-valent vaccine.

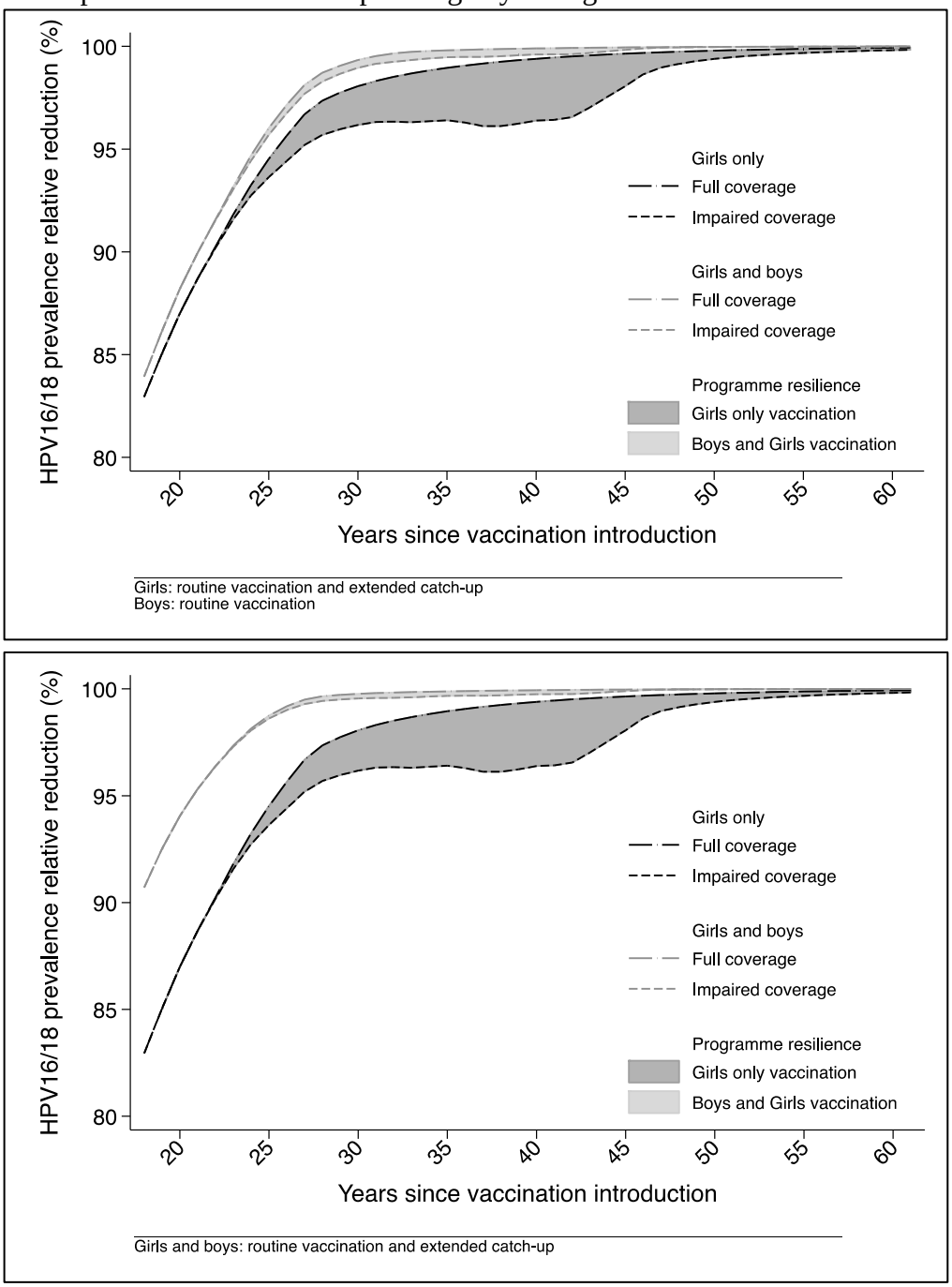
Figure 8. Percent prevalence reduction attributable to vaccination (RAV) of HPV16/18 prevalence in women age 15-34 years



For the first time, we evaluated HPV vaccination program resilience, or the ability of the program to maintain effectiveness despite threats to coverage levels. This was examined by halving the coverage for a period of 5 years and comparing the %RAV. If only girls were

vaccinated, the effectiveness decreased up to 3.1% whereas if boys were included in the vaccination program, either as routine vaccination or routine with an extended catch-up, the decrease in effectiveness was negligible (peak reduction was 0.43%) (Figure 9).

Figure 9. Resilience of vaccination strategies to a 5-year impaired coverage
Upper panel: extended catch-up among girls only
Lower panel: extended catch-up among boys and girls



The stability of a vaccination program is dependent upon healthcare politics including budgets and priorities and acceptance of the vaccine in the population. Dramatic decreases in coverage have been observed in other countries where faith in the vaccine dropped suddenly (133). Drops in effectiveness would have long-lasting effects, especially if there is a change in the

background infection rates as a result of changes in risk-taking behavior, migration in the population, or if screening programs change as a result of vaccination and can no longer accommodate unvaccinated or under-vaccinated cohorts.

Instead of calculating cost-effectiveness of different strategies, we opted to compare the cumulative number of doses needed in Strategies 2, 3, and 4 to the base-case Strategy 1. The results are based on demographic projections and therefore aim to avoid pitfalls inherent in basing cost calculations on quickly changing vaccine prices. As expected, given the scope of the expanded catch-up strategies proposed, compared to the base-case, Strategies 2, 3, and 4 required an increased number of doses, especially during the implementation of the catch-up. After 30 years, the ratio of cumulative number of doses used converged to 1 and 2 times the base-case for Strategies 2 and 3; however, it took nearly 50 years for Strategy 4 to converge to 2 times the base-case. With declining vaccine prices, extending vaccination to more cohorts is attractive; programs will need to consider the increases in doses needed compared to existing efforts to be sure health budgets and systems will cope.

7.6 METHODOLOGICAL CONSIDERATIONS

We are not afraid to follow truth wherever it may lead, nor to tolerate any error so long as reason is left free to combat it.

-Thomas Jefferson

There are a series of methodological considerations that warrant discussion. While we have tried to address the major issues in the articles themselves, some concepts deserve further attention.

Misclassification is a type of measurement error and can be divided into differential, where the probability of being misclassified differs by group or category, and non-differential misclassification, where the probability of being misclassified is the same across groups or categories (50). In cervical screening, misclassification of disease outcomes occurs as a result of the screening (sample taking) or the laboratory interpretation (134). In studies II and III, there is a chance that the cytological and histopathological endpoints used could be misclassified. Most likely the misclassification would be non-differential as the outcomes were collected from the NKCx and diagnoses were determined in routine health care without knowledge of randomization group. To overcome this, all endpoints could have been re-reviewed. However, this was unfeasible in the current studies. Furthermore, if this had been carried out, the results would not be as directly representative of diagnoses found in the screening.

An improvement to the original HPV test was used for the testing of frozen baseline samples in the control arm of Swedescreen (data used in Studies II and III). This improvement resulted in some increase in HR HPV prevalence in the control arm (9.3%) as compared to the intervention arm (7.3%). However, in Study II we found that the increased sensitivity of the updated test did not result in a difference in longitudinal sensitivity until after 5 years of follow-up. When the Swedescreen trial was designed, IARC classified 14 HPV types as high risk.

Since then, type 66 has been demoted and is no longer classified as oncogenic by IARC. For these analyses, we kept the original HR HPV types included at the beginning of the study. We present type-specific and adjusted measures allowing the readers to evaluate the risk evidence on type 66. It should be noted that we have chosen the term "non-HR HPV type" instead of "low-risk HPV type" as a more conservative approach to describing oncogenic potential. Definitively calling types "low-risk" is a potential misnomer as we have not concluded that they cannot cause cancer, only that it has not yet be definitively observed.

The Swedescreen RCT trial did not systematically collect information on further background characteristics or behavior of the study participants. The study was randomized and women in the groups were found to be similar. Studies including behavioral risk factors are important for understanding risk factors for disease. However, the Swedescreen trial aimed to evaluate the impact of screening with different methods. There may come a time when screening is stratified more closely by individual risk profiles, such as vaccination status, but for the time being, the program seeks to target all women, regardless of sexual behavior history and STI diagnoses, smoking status, etc.

The external validity of RCTs has been questioned as inclusion criteria for trials are often strict and study settings and protocols may not match real-life healthcare practice (135). Inclusion criteria for the European RCTs included in this thesis were simple and mainly related to characteristics that would have excluded women from routine screening anyway. Women were recruited from organized screening programs and are therefore representative of women attending screening; however, they may be different from women who do not routinely attend in terms of risk for cervical cancer.

Heterogeneity between studies was a concern in Study I given somewhat different study designs and follow-up strategies. That said, study specific estimates of the effect of HPV screening compared to cytology screening were similar before pooling, suggesting that differences between studies did not have a major impact on the overall impact the results. Tests for heterogeneity revealed that there were no significant differences in the study materials and the random effects models gave similar results to the fixed effects models. There was heterogeneity detected when examining rate ratio for women who had a biopsy in the intervention and control arms; however, the heterogeneity between the remaining studies disappeared when NTCC was removed from the pooled estimates.

The questionnaire circulated for Studies IV and V was designed early in my PhD career and was a first attempt at capturing detailed program information. After reviewing the data sent in by countries and through discussing the results and working through reviewers comments, there are several areas of improvement and a series of additional questions that could be added to further describe program nuances. The results presented in Studies IV and V serve as a baseline description of prevention efforts in Europe and the plan is to update and circulate the questionnaire at regular intervals so that changes to programs can be captured.

Given the political nature of the survey, countries may have felt pressured to present progress towards implementing organized, quality-assured prevention programs in a more

positive light since international recommendations exist. The results may be impacted by some reporting bias, or under-reporting of issues that need to be improved. The language and terminology used in the survey was intended to mirror existing literature and guidelines but concepts could be defined differently across countries leading to variations in question interpretations. We did not explore prevention efforts in countries that reported not having an organized program. Perhaps screening and vaccination efforts in these countries should have been explored in more depth so that as healthcare systems further develop and adopt new policies, comparisons could have been made and impact of changes to screening practice more closely monitored.

With regard to specific changes that could be addressed in subsequent rounds of the survey to better capture screening information, additional information on how organized programs track tests taken outside the program would have been useful. For example, what proportion of tests taken outside the program could be classified as over-screening and how do programs calculate the proportion of tests taken outside the program? Individual invitations to screening are known to have an effect on coverage but invitations systems are reliant on up-to-date and accurate information on the target population. More information on the status of personal data laws would have been helpful to provide context for invitations and data collection possibilities. Furthermore, for programs that can integrate opportunistic and organized tests, we could have asked more systematically whether organized test invitations are delayed as result of an opportunistic test and how exclusion criteria for invitations are verified (e.g. hysterectomies). We asked if staff responsibilities were defined within the screening program but we did not go on to ask more specific questions about individual responsibilities of program actors. This could be included in the next round of the survey. Collecting information on costs was challenging as healthcare systems are country-specific and, depending on integration of screening in health care, direct medical costs may be difficult to tease out from other services. That said, more information on where financing comes from for different parts of the screening process could have been interesting. As more countries implement primary HPV-based screening, questions will need to be added to capture the specifics of HPV screening organization and QA.

The survey did not fully address issues of QA in vaccination programs. To better describe QA in vaccination programs, further questions could have been asked about the vaccine cold chain, vaccine delivery systems, timing of the vaccine schedule, and follow-up of vaccine safety issues including vaccine administration. The information collected pertains more to the overall monitoring and evaluation of vaccination programs and whether there is a link between vaccination and screening. We asked countries to submit information on how they calculated vaccination coverage including how they accounted for schedules that extended over reporting periods and receipt of doses outside the recommended schedule. In the next iteration of the survey, the connection between vaccination delivery strategy and impact in population could be further investigated and more specific information on coverage trends and compliance to dosing schedules could be collected.

For the model, we decided to focus on the HPV types in the current vaccines, modeling the 9-valent results in the additional analyses. Cross-protection of the vaccines was not modelled given uncertainties in the long-term duration of the cross protection (136). We also did not look at outcomes among men as the natural history of infections in men is less well-defined. There has been a significant movement towards modeling the cost-effectiveness of HPV vaccination; however, outcomes and cost modeling approaches differ (137, 138) and input healthcare and vaccine costs change. We chose to focus on a ratio measure of the cumulative number of doses where each alternative vaccination strategy was compared to the base case.

In contrast to non-communicable diseases, when dealing with a communicable disease, intervening to prevent infection or disease in some will have repercussions for others (139). Dynamic transmission models are well-suited for capturing the impact of HPV vaccination as they allow for infection risk to change over time as a result of herd protection. HPV-related outcomes have been modeled using a natural history approach as well. In this case, model compartments represent different health states and movement between the states is based on a probability (140). The choice of modeling technique in Study VI reflects the desire to represent HPV as a communicable infection and to account for indirect effects of prevention strategies. Other modeling approaches may also be valid. As the number of HPV models increase, comparing results becomes increasingly difficult as structures and assumptions differ. This has an impact on the likelihood that model results will be translated into public health practice as tradeoffs might not be clear or applicable across settings. There is an international group of modelers working to standardize HPV model reporting by developing a framework similar to the CONSORT guidelines which bodes well for continued transparency and quality.

8 CONCLUSIONS

Studies I, II, and III

- Compared to screening with cytology, HPV-based screening provides 60-70% greater protection against invasive cervical cancer, although differences by histological type were found.
- HPV-based screening can effectively start at age 30, and screening intervals can be lengthened to at least 5 years.
- The longitudinal sensitivity of cytology for high grade lesions at 3 years of follow-up was similar to the sensitivity of HPV testing at 5 years of follow-up.
- The cumulative incidence of high grade lesions was the same for HPV- and cytology screening, implying that the increased sensitivity of HPV screening reflects early diagnosis rather than overdiagnosis.
- The type-specific IRRs for ASCUS/LSIL were high in the first screening round but decreased over subsequent screening rounds.
- HPV type 16 contributed to the greatest proportion of ASCUS, LSIL, and CIN1 risk in the population.
- Most ASCUS/LSIL lesions are caused by new HPV infections and lesions are most often found in the first screening round.

Studies IV and V

- Organized efforts for QA, monitoring, and evaluation in cervical screening were carried out to a differing extent and were not standardized, making it difficult to compare prevention efforts.
- Most countries found it hard to estimate the costs associated with launching and operating the screening organized program.
- The majority of European countries had some level of HPV vaccination activity; however, organization and quality differed across countries.
- Centralized vaccine registries were in place in the majority of countries with an organized program, allowing for monitoring of key indicators but level detail varied.
- Costs of organization and monitoring were difficult to estimate and varied significantly.
- Further development of this survey tool could support ongoing evaluation of prevention program development.

Study VI

- Extending vaccination catch-up of women and men would further reduce HPV prevalence in women, achieving results earlier than female-only routine vaccination
- The resilience of vaccination programs is improved by including males in the vaccination program.

9 IMPLICATIONS AND FUTURE DIRECTIONS

Evidence has amassed from randomized controlled trials comparing HPV- and cytology-based screening demonstrating that HPV-testing is effective as a primary screening tool. The movement towards primary HPV-based screening has now begun. A pilot study is on-going in the context of the Stockholm County organized screening program where women have been randomized 1:1 to HPV- or cytology-based testing. The pilot program will be complete before Sweden as a whole moves to HPV-based screening in 2017. A pilot program to evaluate HPV-based screening in population is also on-going in England and in the Netherlands, a decision was made to switch to HPV-based testing for the screening program in 2016. The results of Studies I and II imply that intervals for HPV-based screening could be lengthened. However, actual intervals for proposed HPV-based screening schemes differ – in Sweden the proposal includes 3 and 7 year intervals for younger and older age groups, respectively, and in the Netherlands 10-year intervals will be used. Switches in testing method will need to be monitored and evaluated. Most likely, additional adjustments will need to be made to incrementally optimize new strategies.

The EU guidelines from 2008 recommended that HPV testing as a primary screening method should be used only in pilot programs (53). Updated cervical cancer screening guidelines are due to be released shortly. As preliminarily released at the EUROGIN International Congress in Spain this past February, the updated guidelines will recommend that primary HPV-based screening can be implemented context of an organized, population-based screening programs with developed guidelines for triage, referral, and follow-up testing of positive women. Co-testing with cytology will be discouraged, in-line with our results from Study II. The updated guidelines will recommend the use of cytology as triage and caution against referring all HPV-positive women to colposcopy. Programs will need to budget time for counselling HPV-positive women as HPV-test results can carry different implications than cytology and raise questions regarding STIs, transmission, and management strategies.

Organized prevention efforts are favored as they provide a structure for monitoring and evaluating program efforts and can ensure more equitable access to care and resources. In Studies IV and V, we mapped and tried to examine nuances in prevention efforts in order to better understand country-specific health systems and, over time, guide changes that need to be made to strengthen guidelines and prevention efforts. In the next iteration of the survey, greater focus will need to be placed on gathering information regarding shifts in testing methods and screening guidelines for vaccinated populations. Changes will occur rapidly once the updated guidelines are released so recirculating the survey would be advantageous to capture policy shifts and impact.

Since the introduction of the HPV vaccination into routine vaccination programs, the target gender of vaccination has been intensely debated. In Study VI, we show that extending vaccination catch-up among girls and including boys in vaccination programs anticipates reductions in prevalence among girls, achieving results earlier, and strengthens the resilience of programs. The resilience of vaccination programs is of particular concern in settings

where healthcare stability cannot be guaranteed. Fluctuations in vaccination coverage appear to have a long-lasting impact on program effectiveness. When we modelled a complete interruption of vaccination, the effectiveness of girls-only vaccination against vaccine-type HPV prevalence dropped significantly compared to when both girls and boys were vaccinated. Gender neutral vaccination could be considered as a tool to ensure that temporary coverage issues cannot threaten prevention efforts but settings that could benefit the most from extending vaccination may have the greatest challenges in securing consistent funding for vaccines.

While the effectiveness of the HPV vaccine decreases with increasing age at vaccination, older girls and women may still benefit from vaccination (83). Vaccination up to 26 is covered by Stockholm County, and with this in mind, we are working to organize a catch-up of girls between the ages of 19 and 26 in the hopes of increasing coverage in this older age group. This will also allow us to evaluate the performance of screening post-vaccination. By vaccinating older girls and screening with HPV testing, the infections we detect will most likely be persistent infections, increasing the predictive value of HPV testing in younger women.

Vaccine efficacy in males has be demonstrated in trials (141, 142) and some countries – Austria, Australian, and the United States – have started to encourage male vaccination (94, 95). Implementing HPV vaccination of boys would require at least doubling the number of doses currently used, and more if extended catch-up programs were also included. Issues of ethics also come into play when considering the target gender of HPV vaccination – is it ethical to require that females bear the burden of vaccination when HPV infection effects and can be transmitted by both genders? Taking a broader perspective, are we missing potentially high-risk populations that will not benefit from herd effect generated by female-only vaccination? Evidence suggests that rates of anal cancer are high among men who have sex with men (MSM) (143) but female-only vaccination programs will not benefit MSM (144) and pockets of unvaccinated populations could be reservoirs for infection (145). As costs for the vaccines decrease and awareness of HPV and HPV-related diseases increases, hopefully the choice between which gender(s) to vaccinate will become less controversial.

HPV DNA testing can be implemented as a self-test and has been shown to increase screening uptake among non-attenders to screening (146). While evidence suggests that clinician-collected samples have better sensitivity than self-collected samples, using a PCR-based HPV test can improve the relative sensitivity and specificity of self-collected samples (147). Rapid HPV tests, such as careHPV, have recently been evaluated in resource-poor settings and promise better sensitivity than cytology and visual inspection methods which rely on more subjective evaluation of samples (148). With an increasing number of tests available on the market and progressively more complex screening and triage algorithms, the search for the appropriate use of biomarkers in determining risk has become an area of growing attention. Specifically, an area of particular interest is the possibility of stratifying HPV positive women by risk for progression to lesions using biomarkers of gene over expression (149). If these biomarkers prove to be effective, then a new age of even more individualized, tailored screening is possible. A balance would have to be found that allows

for a logistically viable generalized implementation and meaningful, individualized risk stratification. Programs must be able to maintain high coverage and must further develop outreach to long-term non-attenders.

For many years to come, we will have non-vaccinated cohorts, partially vaccinated cohorts, and vaccinated cohorts all participating in screening with varying degrees of risk for cancer. Next generation vaccines, such as the 9-valent vaccine protecting against HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58, promise extended protection against cervical cancer (80). As the first cohorts of women vaccinated enter screening ages, screening programs will need to adjust, especially with the prospect of vaccines that include an increasing number of types. The decrease in cancer risk in the population after mass-vaccination and the decrease in CIN2+ risk in HPV-positive women will lead to a different trade-offs between screening efforts and benefits. The intersection of vaccination and screening efforts needs to be modelled and evaluated so that we make the most efficient use of healthcare resources and testing methods while promoting the health of our populations (150).

As we move from trial evidence to practice, focusing our energy on evaluating prevention strategies in population using a comparative effectiveness research (CER) design, also known as randomized health services (RHS) trials could help to inform the broader roll-out of new policies. Evidence from randomized controlled trials have provided strong efficacy results HPV vaccines and screening methods. The next step is to implement new, proven prevention strategies through the routine health service infrastructure. RHS trials are designed to implement new strategies in a randomized fashion in order to reduce potential for bias and ensure that the outcomes evaluated are temporally comparable (151). Such trials can help us understand whether the new intervention is cost-effective, has the effect anticipated, is accepted in population, and whether the health system infrastructure can adapt to new policies and procedures (151). In a RHS design, costs of the new intervention and related services are carried by the health system itself or whatever local routine is applicable (ministry of health, individual-payer etc) but the results are analyzed in a research setting and the study is submitted for ethical review.

Rolling out new interventions with monitoring and incremental revision allows for evidence-based improvement of prevention programs and connects healthcare practice to research. In my experience, the RHS design lends itself to close collaboration between researchers and program officers. Time, effort, and resources can be concentrated on sequential optimization instead of rigid sweeping changes that may not align fully with actual prevention program logistics and may take too long to develop and implement for populations in need.

This thesis has focused on the optimization of cervical cancer efforts in Sweden and the rest of Europe. At the end of the day, Swedish and European women have benefitted tremendously from screening and vaccination. Cervical cancer incidence and mortality have decreased significantly since the implementation of screening and efforts to organize screening and implement HPV vaccination are on-going in most countries. Worldwide, there are approximately 528,000 new cases of cervical cancer each year and 266,000 deaths due to cervical cancer each year, 87% of which occur in resource-poor settings (152). Given the

pressing burden to healthcare systems in the world's poorest countries for treatment of disease, disease prevention efforts cannot always be prioritized. In our efforts to strengthen prevention efforts here at home, we must not forget our sisters in other, less fortunate settings. We must push ourselves to consider the implications of our findings in a variety of settings and continuously lead by example with evidence-based healthcare decision-making. We are fortunate to have a healthcare system and databases to monitor and evaluate healthcare practices and, therefore, I believe that we have a certain obligation to lead in seeking answers to some of the remaining questions.

10 ACKNOWLEDGEMENTS

To my supervisors, who took a chance on the application from the States. Thank you for being my constellation of knowledge, support, and inspiration. I really didn't know what I was embarking on when I came, but through this PhD I've found my purpose and have been given a chance to grow and channel my commitment to public health. **Lisen** – thank you for looking out for me and supporting me as a friend and supervisor. I especially appreciate your honesty, your clarity, and your commitment to ensuring that this experience was an education for me. **Joakim** – thank you for trusting me with your projects and pushing me to think more critically, write more clearly, and work more effectively. I hope that I can emulate the energy and commitment you have for research. **Pontus** – thank you for always being ready for bigger conversations, for being there at critical moments, and encouraging me to dig deep and reflect on what I want.

To the movers and shakers at MEB – thank you to **Camilla Ahlqvist** for all that you do for us students throughout our time in the department. To **Henrik Grönberg** and **Paul Lichtenstein** for your leadership of our department and ensuring that we have the space and resources for our work. To **Ann Almqvist** for your expert help in navigating EU grants. To all former and current students at MEB for lively lunch conversations, stimulating seminars, and research experiences shared – **Donghao Lu, Fei Yang, Hannah Bower, Johanna Holm, Tracy Peters, Thang Trinh, Tong Gong,** and **Vilhelmina Ullemar**. To the members of the LifeGene/BBMRI corridor for fikas, conversation, and a positive work atmosphere, especially **Ann-Sofie Lundin, Jessica Pege, Loreana Norlin, Mariam Lashkariani,** and **Roxana Merino Martinez.**

To my research group and colleagues engaged in cervical cancer prevention – **Pär Sparén** and **Bengt Andrae**, for your abiding faith in us students, for including me in discussions and conferences, and for your help and encouragement through the years. Thank you to **Carina Eklund**, **Vitaly Smelov**, and **Maria Hortlund** for patiently introducing me to the Swedescreen data and for friendship and collaboration. **Kristina Elfgren** – for conversations over the years that have mirrored my commitment to women's health, and connected me to clinical practice. **Eva Herweijer** – you've been there since the very beginning and I will always grateful to you for being my thought-partner, for your friendship, and for how well you know me. To **Favelle Lamb** for your friendship, care, and energy – thank you for making me feel included in the family. To **Jiangrong Wang**, for your curiosity, your thorough approach to research and many wonderful meals together. To **Amy Leval** and **Karin Sundström** who have given me tremendous examples to follow, for your commitment to research, travels together, and pep talks over the years.

A special thank you to **Anna Johansson** for careful and thorough biostatistical assistance and thoughtful teaching and **Fulvio Lazzarato** for patiently teaching me the model. To our broader group of researchers, students, and collaborators: **Alex Ploner, Ellinor Östensson,**

Jiayao Lei, Inga Velicko, Maria Persson, Ninoa Malki, Olof Grönlund, Peter Olausson, Pouran Almstedt, Sara Fogelberg, Sara Nordqvist Kleppe, **Sonia Andersson** and **Tove Edsbäcker**. A special thank you to **Lawrence von Karsa** and **Sven Törnberg** for introducing me to the European prevention community at ESSM and grounding me in the practice of screening.

I am grateful to have participated in two EU FP7 Program grants where I had the opportunity to work with renowned researchers and incredible data. I am especially grateful for my collaboration with **Iacopo Baussano**. Thank you for teaching me modeling and pushing my thinking. And, thank you for all the conversations between meetings and at conferences that have helped me approach my studies and pushed me to own my learning process. Thank you to **Matti Lehtinen** who has included me since my very first weeks as a PhD student.

To the MEB-gäng – thank you for welcoming and including me when I was completely new and for being a steady circle of friendship and support. Thank you to **Adina Feldman** for friendship, travels, and celebrations shared; **Lisa Möller** for always carefully listening and giving encouragement; to **Maria Sandberg** for leading the way in combining research and practice; to **Alexander Grankvist, Anders Carlson, Björn Schelin, Bruno Raposo, Fredrik Havasi, Iffat Rahman, Karin Sundström, Lovisa Högberg, Martin Fransson, Mats Ljung, Sara Christensen, Stephanie Bonn, Thomas Frisell, Therese Ljung** for your wit, care, and warmth over the years.

To **Carolyn Cesta** and **Anne Örtqvist** – for meeting me on so many different levels: for training, travels, breakfasts, and research ponderings shared. For thoughtful conversations about careers and life and for the constancy of your care and support, especially in this last sprint to the finish.

To the expat running group – **Eva Herweijer**, **Daniela Mariosa**, **Diana Muessgens**, **Miriam Mosing**, **Kelly Shaffer**, and **Megan Spencer-Smith**. Thank you for all the evenings we've had together, for friendship, celebrations, races, and travels shared. To **David Nachtweih** and **Shea Kluender** and my fellow expat **Sarah Horsch** – from bright eyed associates in NYC to new parents, you have taught me a lot about who I want to be and how to get there.

To the book club – for making sure that I read things unrelated to cervical cancer, for lively discussion and friendship – **Ralf Kuja-Halkola, Robert Karlsson, Therese Ljung,** and **Thomas Frisell.**

To **Katie Zmijewski** and **Sofie Petersson** for being my first Swedish friends – for always making me feel included, for bravely discussing life's big questions, and for your positive outlook.

To my friends and colleagues from Emory where I had the fortune to begin my work in

public health. **Amy Winter**, **Kristen Little**, **Rob Stephenson**, **Deb McFarland**, **Roger Rochat**, and **Mo Ali** who approach their research with a deep commitment to seeking knowledge and dedication to public health.

To **Ann-Sofie** and **Lasse** – for opening your home to me and including me in grand skiing and sailing adventures. Your warmth and attention have been especially meaningful to me as I have worked to build a life for myself here.

To my godparents on both sides of the ocean, **Tom**, **Kathy**, **Åsa** and **Swen**. Thank you for always making a special place for me in your lives, for marking my milestones and reminding me of your care and love.

To my oldest and dearest friend **Laila Füchtbauer** – thank you for your constancy, your humor, and your deep kindness and wisdom. I am forever grateful that we have continued to be in each other's lives despite distance and time. Thank you to **Anders Larsen**, for your friendship and warmth as we've gone through this PhD process in parallel. To **Johannes**, who reminds me to slow down and joyfully appreciate small details.

To **Lotta Nordström**, **John Lamb**, **Lisa Niklasson**, **Jörgen Niklasson**, and **Ingrid Schmidt** for welcoming and including me from the very beginning. Thank you for being my family here in Stockholm, for meals, travels, and cozy time at home together.

To my extended family – to my strong aunts **Marit**, **Bodil**, **Mary**, and **Margie**, thank you for teaching me to never question my strength as a woman. Even though we've always been separated by distances, I have felt your support and encouragement. To my uncles **Gunnar** and **Göte** – for your abiding interest in my life and thoughtful conversations. To my **cousins** and **second cousins** spread across the US, Norway, and Sweden, and **grandparents** for giving me a strong sense of family and where I come from.

To my parents, my brother **Manfred**, my sister **Madelene**, and my sister-in-law **Freya**. **Mom** and **Pop** – thank you for your incredible investment in our education and thank you for always encouraging us to find our individual calling. Thank you for a childhood and a home that opened the world to us. To my siblings – thank you for giving me living examples of what it means to follow your convictions. I miss you when we're so far away from each other but I am grateful that modern technology allows sharing updates, jokes, and life's adventures by Facetime and Skype in China, Canada, rural Georgia, Falls Church, and Washington State.

To my partner, **Jonas**, thank you for our home and our life together. Thank you for being there each step of the way and unconditionally supporting me; for understanding my commitment to work and sharing my enthusiasm for life. Yay!

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12 APPENDIX

12.1 EUROPEAN UNION SURVEY ON ORGANIZATION AND QUALITY CONTROL OF CERVICAL CANCER SCREENING AND HPV VACCINATION PROGRAMS