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PREVENTION AND PROGNOSIS OF CERVICAL CANCER: THE INTERPLAY OF HUMAN PAPILLOMAVIRUS, VACCINATION AND SCREENING

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Prevention and prognosis of cervical cancer: the interplay of human papillomavirus, vaccination and screening
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To my beloved family

致我最亲爱的爸爸妈妈

ABSTRACT

Human papillomavirus (HPV) is the major cause of cervical cancer. The well-established natural history from HPV infection to the occurrence of invasive cervical cancer serves as the basis for prevention of cervical cancer through prophylactic HPV vaccination (primary prevention) and cervical screening (secondary prevention). Cervical cancer detected through screening also has better chances of being cured than cancers not detected through screening. This thesis addresses research questions on prevention and prognosis of cervical cancer within the framework of the interplay of HPV, vaccination, and cervical screening, and it also provides insights for evidence-based decision-making.

In **Paper I**, we examined the association between cervical screening with cytology and risk of adenosquamous cell carcinoma (ASC) and rare histological types of invasive cervical carcinoma (RICC). Based on a nationwide cervical cancer Audit, we conducted a nested case-control study including 338 cases of ASC and RICC diagnosed during 2002-2011 in Sweden with their year-of-birth-matched controls. We found that screening with cytology was associated with decreased risk of ASC and RICC, but the magnitude of risk reduction in relation to cervical screening was less for RICC than for ASC. The majority of ASC and RICC cases were positive for high-risk HPV in tumor tissues.

In **Paper II**, we evaluated whether the tumor high-risk human papillomavirus (hrHPV) status was associated with the prognosis of cervical cancer. In a nationwide population-based study, we included 2845 primary invasive cervical cancer cases diagnosed in Sweden during 2002–2011, and comprehensively tested diagnostic blocks for 13 hrHPV types and 24 HPV types which were not established as oncogenic. Women with hrHPV-positive cervical tumors had a substantially better prognosis (39% lower excess mortality) than women with hrHPV-negative tumors. The difference of prognosis by tumor hrHPV status remained statistically significant, irrespective of age, cancer stage, and histological type.

In **Paper III**, we investigated screening performance in terms of positive predictive value (PPV) of cytology for cervical intraepithelial neoplasia grade 2 or worse (CIN2+) among HPV-vaccinated birth cohorts. Using a population-based cohort design, we included women born 1989-1993 who were resident in Sweden since the introduction of HPV vaccination and attended cervical screening at age 23, based on records from Swedish National Cervical Screening Registry (NKCx). We found that vaccinated women had lower PPV of cytology for CIN2+ compared to unvaccinated women. The decrease in PPV was greater among women vaccinated before age 17 than those vaccinated at age 17-22.

In **Paper IV**, we assessed the association between HPV vaccination and the risk of invasive cervical cancer in a population-based cohort study. An open cohort of women aged 15-30 (including age 30) living in Sweden were included and followed during 2006-2017 for HPV vaccination and first occurrence of invasive cervical cancer. The findings showed that HPV vaccination was related to a 52% lower risk of invasive cervical cancer for vaccinated women

compared to unvaccinated women. Younger age at vaccination initiation was associated with a more pronounced risk reduction (83% risk reduction when vaccinated before age 17).

In conclusion, this thesis shows that cervical screening can effectively reduce the risk of ASC and RICC, which is beyond the established evidence on preventing squamous cell carcinoma and adenocarcinoma. HPV vaccination can effectively reduce the risk of cervical cancer; the ultimate goal of cervical cancer prevention. With the implementation of HPV vaccination, the PPV of cytology for CIN2+ has decreased in vaccinated women compared to unvaccinated women, especially among those vaccinated at younger age. Tumor hrHPV status is associated with the prognosis of cervical cancer, which could add value to the clinically established prognostic factors. Taken together, these studies add knowledge to the current understanding of cervical cancer prevention strategies and prognosis of cervical cancer, and serve as a basis for evidence-based decision-making and policy changes in the future.

LIST OF SCIENTIFIC PAPERS

I. Lei J, Andrae B, Ploner A, Lagheden C, Eklund C, Nordqvist Kleppe S, Wang J, Fang F, Dillner J, Elfström KM, Sparén P. Cervical screening and risk of adenosquamous and rare histological types of invasive cervical carcinoma: population based nested case-control study. BMJ. 2019 Apr 3;365:l1207

II. Lei J, Ploner A, Lagheden C, Eklund C, Nordqvist Kleppe S, Andrae B, Elfström KM, Dillner J, Sparén P, Sundström K. High-risk human papillomavirus status and prognosis in invasive cervical cancer: A nationwide cohort study. PLoS medicine 2018 15;10 e1002666

III. Lei J, Ploner A, Lehtinen M, Sparén P, Dillner J, Elfström KM. Impact of HPV vaccination on cervical screening performance: A population-based cohort study. (Submitted)

IV. Lei J, Ploner A, Elfström KM, Wang J, Roth A, Fang F, Sundström K, Dillner J, Sparén P. Effectiveness of HPV vaccination against invasive cervical cancer: A population-based cohort study. (Submitted)

RELATED PUBLICATIONS

(Not included in the thesis)

- Lagheden C, Eklund C, Lamin H, Kleppe SN, Lei J, Elfström KM, et al. Nationwide comprehensive human papillomavirus (HPV) genotyping of invasive cervical cancer. Br J Cancer. 2018;118(10):1377-81.

- Wang J, Elfström KM, Andrae B, Nordqvist Kleppe S, Ploner A, Lei J, et al. Cervical cancer case-control audit: Results from routine evaluation of a nationwide cervical screening program. Int J Cancer. 2019.

- Arroyo Muhr LS, Lagheden C, Eklund C, Lei J, Nordqvist Kleppe S, Sparén P, et al. Sequencing detects human papillomavirus in some apparently HPV-negative invasive cervical cancers. J Gen Virol. 2019.

CONTENTS

LIST OF ABBREVIATIONS

AC	Adenocarcinoma
ACCES	Advancing Cervical Cancer Eradication Strategies
ASC	Adenosquamous cell carcinoma
ATC	Anatomical Therapeutic Chemical
CIN2+	Cervical intraepithelial neoplasia grade 2 or worse
CIN3+	Cervical intraepithelial neoplasia grade 3 or worse
CI	Confidence interval
DNA	Deoxyribonucleic acid
EHR	Excess hazard ratio
EMA	European Medicines Agency
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
FIGO	International Federation of Gynecology and Obstetrics
HPV	Human papillomavirus
hrHPV	High-risk human papillomavirus
IARC	International Agency for Research on Cancer
ICD	International Classification of Diseases
ICD-O-3	International Classification of Diseases for Oncology, 3rd Edition
IRR	Incidence rate ratio
IR	Incidence rate
LISA	Longitudinal integration database for health insurance and labour market studies
MGR	Multi-Generation Register
mRNA	Messenger ribonucleic acid
NBHW	National Board of Health and Welfare (Socialstyrelsen)
NKCx	Swedish National Cervical Screening Registry
NPV	Negative predictive value
NVR	National Vaccination Register
OR	Odds ratio
PCR	Polymerase chain reaction
PIN	Personal identity number
PPV	Positive predictive value
RICC	Rare histological types of invasive cervical carcinoma

RR	Risk ratio
RSR	Relative survival ratio
SCB	Statistics Sweden (Statistika Centralbyrån)
SCC	Squamous cell carcinoma
SCR	Swedish cancer register
SNOMED	Systematized Nomenclature of Medicine
STI	Sexually transmitted infection
SVEVAC	Swedish HPV Vaccination Register
TPR	Total Population Register
WHO	World Health Organization
VLPs	Virus-like particles

1 INTRODUCTION

Human papillomavirus (HPV) was discovered in 1907, and a possible link between HPV infection and cervical cancer was suggested by Dr. zur Hausen in 1974 (1). HPV16 was first detected in a cervical cancer patient in 1983 (2). Thereafter, the causal relation between HPV infection and cervical cancer has been established (3, 4). Interestingly, the Papanicolaou test (abbreviated as Pap test, and also called "Pap smear"), was first presented by Dr. Papanikolaou in 1928 describing as a new diagnostic method for malignant tumors in female genital tract (5), which was long before the discovery of HPV as a causal agent of cervical cancer. The Pap test, validated as a diagnostic tool in 1943 (6), has been used in cervical screening programs for identifying malignancy and cell changes. The discovery of HPV and its etiological link to cervical cancer led to the development of HPV vaccines (7, 8). Randomized controlled trials of prophylactic HPV vaccines were launched in 1991 to test their protection against HPV infection and cervical diseases (9-11). Since 2006, the Food and Drug Administration (FDA) has approved three prophylactic HPV vaccines (12-14).

Cervical screening with Pap smear has been introduced in many countries through either opportunistic or organized screening programs, and substantially reduced incidence and mortality of cervical cancer, especially in countries with an organized screening program (15) and for the major histological types of cervical cancer (16). HPV vaccination was introduced more than 10 years ago and has been shown to have tremendous population impact in reducing incidence of HPV infection and cervical lesions (17). Currently, a transition from cytology screening using Pap smears to HPV-testing as the primary screening test is ongoing, offering greater protection against invasive cervical cancer (18). Cervical cancer has become a preventable disease and we are now in an era with the potential to eliminate cervical cancer (19). However, some unresolved questions of scientific and strategic significance remain for prevention and prognosis of cervical cancer.

This thesis investigates the association between cervical screening, HPV vaccination and risk of cervical cancer, the role of HPV in prognosis of cervical cancer, and how HPV vaccination will impact the screening strategies in practice to inform evidence-based public health policy change and clinical decision-making.

2 BACKGROUND

2.1 HUMAN PAPILLOMAVIRUS AND CERVICAL CANCER

More than 200 types of human papillomavirus (HPV) have been identified (20). HPV is a virus with double-strain deoxyribonucleic acid (DNA), and comprises 8 protein-coding genes and a long control region responsible for regulatory functions (21). The L1 and L2 encode capsid proteins, which are usually expressed at late differentiation stage of epithelium, related to virus assembly and released from the superficial epithelium (22). E1, E2, E4, E5, E6, and E7 encode proteins that contribute to replication, transcription, and transformation (21, 23). Among those, E6 and E7 are two major oncoproteins targeting tumor suppressor p53 and retinoblastoma tumor suppression protein (pRB), respectively. E6 and E7 can alter cellular proliferation, cell-cycle progression, and apoptosis (22, 24). Additionally, they are also involved in viral DNA integration, malignant transformation, and the progression to cancer (22).

HPV is the major cause for cervical cancer (25). HPV infection is the most common sexually transmitted infection (STI); it peaks among women at younger age (below age 25) but decreases afterwards (26). According to a global meta-analysis (27), the prevalence of HPV infection among women who had normal cytology was around 24% for women under age 25. A rebound of HPV infection at older ages (age≥45) in certain regions (Americas and Africa) has been documented (27). The International Agency for Research on Cancer (IARC) has classified HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 as oncogenic types and HPV 68 as probably oncogenic (28). Persistent infections with oncogenic HPV types cause most cervical cancer cases, in which HPV16 and HPV18 account for approximately 70% of all cervical cancer (29). In addition, anal cancer (88.0%), penile cancer (50.0%), vulvar cancer (24.9%), vaginal cancer (78.0%) and oropharyngeal cancer (30.8%) are attributed to HPV infections (30).

Cervical cancer usually occurs in the “transformation zone” of cervix, which is an area in the cervix where columnar cells constantly transform into squamous cells (Figure 2.1). The entire anogenital epithelium can be infected by HPV, but the epithelium in transformation zone is especially susceptible to carcinogenesis and therefore becomes the most common area for abnormal cells to develop in the cervix (31). Squamous cell carcinoma and adenocarcinoma are two major histological types of invasive cervical cancer (25), which account for approximately 75% and 20% of all histological types of cervical cancer, respectively; while other or unspecified histological types constitute the remaining 5% (25). The 5% of uncommon histological types of cervical cancer are usually difficult to classify histologically, and are accepted as a distinct clinical-pathological entity due to their aggressive progression and worse prognosis compared to squamous cell carcinoma and adenocarcinoma (32, 33).

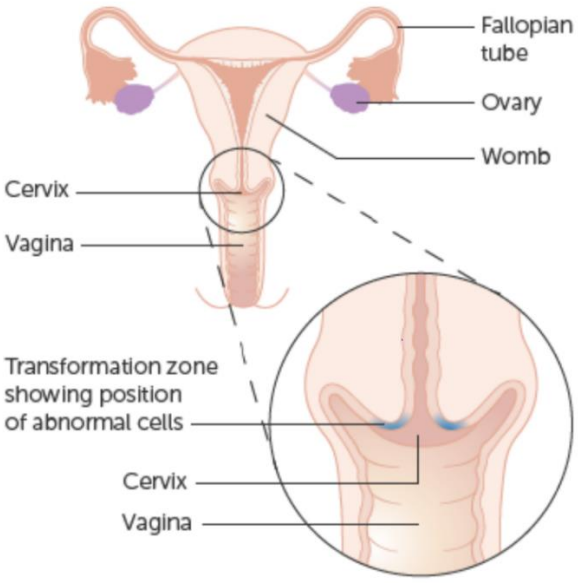

Figure 2.1 *Anatomy of female reproductive system and transformation zone.* Cancer Research UK copyright.

In the natural history of cervical cancer (34) (Figure 2.2), HPV infections can usually be cleared without intervention within a few months, and about 90% of the infections are cleared within 2 years (35). Only a small proportion (10%) of persistent infections with carcinogenic types of HPV develop into precancerous lesions (36), and if left untreated, can progress to invasive cervical cancer. In addition to the major risk factor – HPV infection, other non-viral factors (37) for cervical cancer include current smoking (37), multiparity (38), immunodeficiency, younger age at sexual debut (39), higher life-time number of sexual partners, poor nutritional status as well as long-term use of oral contraceptives (40). All non-viral factors are mainly associated with an elevated risk of HPV infection, persistence, and progression, and therefore increase the risk of cervical cancer.

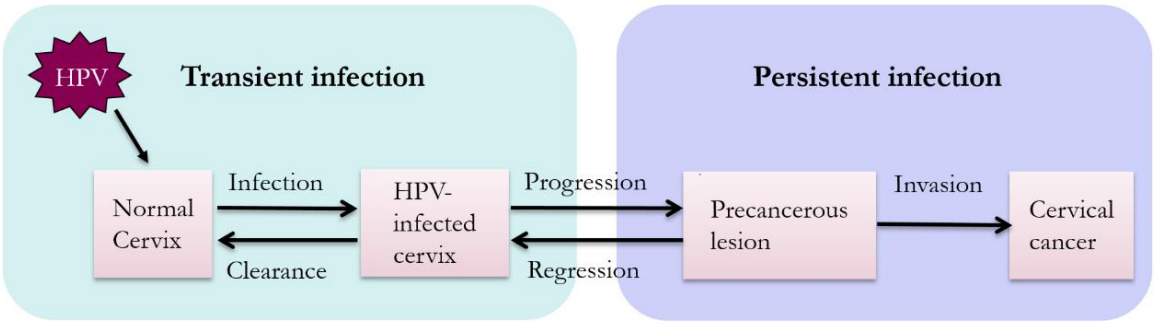

Figure 2.2 *Natural history of cervical cancer.* Adapted from Wright TC Jr et al (34). HPV, human papillomavirus.

2.2 CERVICAL CANCER PREVENTION

The well-established natural history of the development of cervical cancer is the basis for effective preventive procedures. Cervical cancer can be prevented through HPV vaccination (primary prevention) and cervical screening (secondary prevention). Both preventive

procedures aim to interrupt an intermediate step in the chain of the natural history from HPV infection to the occurrence of cervical cancer.

2.2.1 Cervical screening

Cervical screening, secondary prevention, aims to prevent the occurrence of invasive cervical cancer by detecting and removing precancerous lesions, which if left untreated, could develop into invasive cervical cancer (41). There are a variety of cervical screening methods available including conventional cytology, liquid-based cytology, HPV-based screening, and visual inspection with acetic acid or Lugol's iodine. Usage of screening methods is usually dependent on the health care resources and facilities (42). Cervical screening with cytology and HPV-based screening are commonly used in a majority of high- and middle- income countries, while in settings with limited resources, visual inspection with acetic acid or Lugol's iodine could be alternatives. In this thesis, only cytology screening and HPV-based screening will be addressed.

Cervical screening with cytology

- Conventional cytology (Papanicolaou test/Pap smear): which aims to detect precancerous lesions before they develop into cancer through collecting cervical cell samples at the transformation zone and endocervical canal. The collected cervical cells are fixed on a glass slide and examined under the microscope for cell changes or abnormality.

- Liquid-based cytology: the cervical sample is collected in the same way as a Pap smear, however, the sample is immersed into a conserving liquid before being fixed on the slide to reduce the quantity of obscuring material and therefore improve the accuracy. Samples taken in liquid-based cytology can provide representative residual material, which could be used for adjunctive testing (i.e. HPV testing).

HPV-based screening

HPV testing is used to detect whether a woman is infected with high-risk HPV, through detecting the presence of HPV DNA or messenger ribonucleic acid (mRNA) of E6 and E7 oncoproteins in a sample of cervical cells. Samples for HPV-based screening can be collected by healthcare providers or by screening participants themselves with self-sampling kits. Compared to cytology, HPV-testing offers several benefits including higher sensitivity in detecting cervical intraepithelial neoplasia grade 2 or worse (CIN2+) lesions and a higher negative predictive value (NPV) (43). The disadvantage of HPV testing is its lower specificity in detecting CIN2+ (44).

2.2.1.1 Cervical screening programs

Cervical screening with cytology has been successfully implemented in many countries over the past decades. Given the accumulation of evidence on the advantage of HPV-based screening compared to cytology (18, 44, 45), a transition from cervical screening with cytology

to HPV-based screening as the primary screening has been ongoing in recent years. Many countries have issued new guidelines recommending primary HPV screening, however, the screening algorithms, such as age at start of screening and screening intervals, are different. In 2015, the European guidelines (46) for cervical screening recommended HPV testing as the primary method in organized, population-based screening program. In the US, women aged 21 to 29 are recommended to receive 3-year interval cytology test, and women aged 30 to 65 are recommended to receive 5-year interval of HPV and cytology co-test or 3-year interval cytology according to American Cancer Society and American Society for Colposcopy and Cervical Pathology (47). Since December 2017, women aged 25 - 69 years in Australia have been invited to HPV testing at 5-year intervals with partial HPV genotyping for HPV16/18 and an exit test at age 70-74 (48).

In Sweden, cytology screening was introduced in the 1960s. Since 1998, women aged 23 - 60 have been invited to cervical screening with cytology, with a 3-year interval for women aged 23 - 50 and 5-year interval for women aged 51 - 60 according to national guidelines (49). In 2015, the Swedish National Board of Health and Welfare recommended to use primary HPV screening for women age 30 and above because of its superiority in preventing cervical cancer (18, 50). However, the roll-out of primary HPV screening is not nationwide yet. As of August 29th, 2019, 13 out of 21 regions in Sweden have implemented primary HPV screening (51). According to the current guidelines (50), primary screening with cytology is used for women age under 30 at a 3-year interval. Women aged 30 to 49 are invited to HPV screening at 3-year intervals and women aged 50 and above are invited to HPV screening at 7-year intervals. Cytological and HPV-based co-testing is recommended for women age 41. Screening will not stop until women have a negative screening result at least 64 years of age, and invitations continue until age 70 to ensure that women have a final test in the program. Cytology is used as the triage method for all women with HPV-positive results. Women with HPV positive but cytology negative results are subjected to partial HPV genotyping, and they are recommended for repeat HPV testing at 18 months if positive for HPV16 and/or HPV18.

2.2.1.2 Effectiveness of cervical screening

The burden of cervical cancer has been considerably reduced over time after the successful implementation of cytology screening (16, 52), especially in countries with organized cervical screening programs (53, 54). In countries which screening programs that suffer from low quality and coverage, the burden of cervical cancer has remained high (15, 16). In Nordic countries, cytology screening has substantially reduced the incidence (41%-49% less expected cases) of cervical cancer since the introduction of screening (54). Screening additionally contributes to early detection of cervical cancer and down-staging (32, 55), which subsequently improves the chances of being cured (56). In Sweden, it has been estimated that screening prevented almost half of the expected cervical cases between 1960s and 2000s (54). However, an increasing trend of cervical cancer has been observed in recent years (2014-2017), which is probably due to the increased exposure to HPV in younger birth cohorts and reduced sensitivity

of normal cytology test in some laboratories (57). The increase (around 20%) in incidence was mainly observed at ages under 50 and cancers diagnosed at early stage (57).

In terms of effectiveness against different histological types, cytology screening has been found to be effective in preventing squamous cell carcinoma (32, 55, 58), and adenocarcinoma to some extent (55, 59). Evidence on other uncommon types of cervical cancer is absent, aside from the limited evidence on effectiveness of screening against adenosquamous cell carcinoma (ASC) (59, 60). Evaluating screening and risk of uncommon types of cervical cancer has been challenging, considering the limited number of cases, availability of cervical screening information, and the difficulties in classification of histological types. In existing studies, they were usually classified as "non-squamous cell carcinoma", "other types" or simply excluded (32, 59). Over the decades, the incidence of uncommon types varied across countries (49, 53, 61), which could be due to inconsistency in histological classification.

2.2.2 HPV vaccination

The current prophylactic HPV vaccines are adjuvanted non-infectious recombinant vaccines, which are based on virus-like particles (VLPs) produced by recombinant expression of major capsid antigen L1. So far, three HPV vaccines have been licensed by both the FDA and the European Medicines Agency (EMA) to prevent HPV-related diseases that are associated with vaccine types.

The quadrivalent vaccine (Gardasil™, against HPV types 6, 11, 16 and 18) was first available in 2006 (13) followed by bivalent vaccine (Cervarix™, against HPV types 16 and 18) in 2007 (12). The bivalent vaccine and quadrivalent both provide protection against HPV types 16 and 18, which account for around 70% of all cervical cancer. The quadrivalent vaccine also protects against HPV types 6 and 11 that cause around 90% of genital warts (62). In 2014, a 9-valent vaccine which covers 9 types of HPV (HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58) was approved (14), and provided protection against five additional HPV types that are attributable for 20% of cervical cancer (63). All HPV vaccines can be used for both females and males. In 2014, World Health Organization (WHO) recommended a two-dose vaccination schedule considering the non-inferior protection against HPV16/18 infection compared to a three-dose schedule (64). Thereafter, HPV vaccines could be administered either according to a 2-dose schedule or a 3-dose schedule depending on age at vaccination initiation (Table 2.1).

Table 2.1 *Vaccination schedules for prophylactic HPV vaccines.* Source: European Medicine Agency (65-67).

HPV vaccines	HPV types	2-dose, age (schedule)	3-dose, age (schedule)
Cervarix	16, 18	9 to 14 years (6 months apart)	15 years and above (0, 1 and 6 months)
Gardasil	6, 11, 16 and 18	9 to 13 years (6 months apart)	14 years and above (0, 2 and 6 months)
Gardasil 9	6, 11, 16, 18, 31, 33, 45, 52 and 58	9 to 14 years (2^{nd} dose given 5-13 months after the 1^{st} dose)	15 years and above (0, 2 and 6 months)

2.2.2.1 HPV vaccination programs

By 12th December 2019, 124 countries and territories have implemented HPV vaccination in the national immunization programs (68). In Sweden, HPV vaccines were introduced in 2006. Starting from May 2007, HPV vaccines were subsidized for girls aged 13-17. A free-of-charge school-based vaccination program for girls aged 10-12 together with a catch-up vaccination program for girls aged 13-18 was launched in January 2012 (69). Starting in 2015, the Swedish guidelines were updated according to the WHO recommendation and the school-based program began to use a two-dose vaccination schedule for girls 10-12 (grade 5 and 6). Since June 2016, those who did not vaccinate through the school-based program were offered another chance to be vaccinated up to age 18 following a three-dose vaccination plan. Since October 2019, the 9-valent HPV vaccine has been used for the school-based vaccination program.

In Sweden, the vaccination coverage of at least one dose (Figure 2.3) for subsidized HPV vaccination was relatively low and varied from around 35% to below 10% of the target group. However, the vaccination coverage increased to 55-62% for the catch-up program, while school-based HPV vaccination coverage reached 80-86% (70, 71). The two-dose coverage of HPV vaccination was almost 80% for girls born 2002 onwards by the end of 2019 (72).

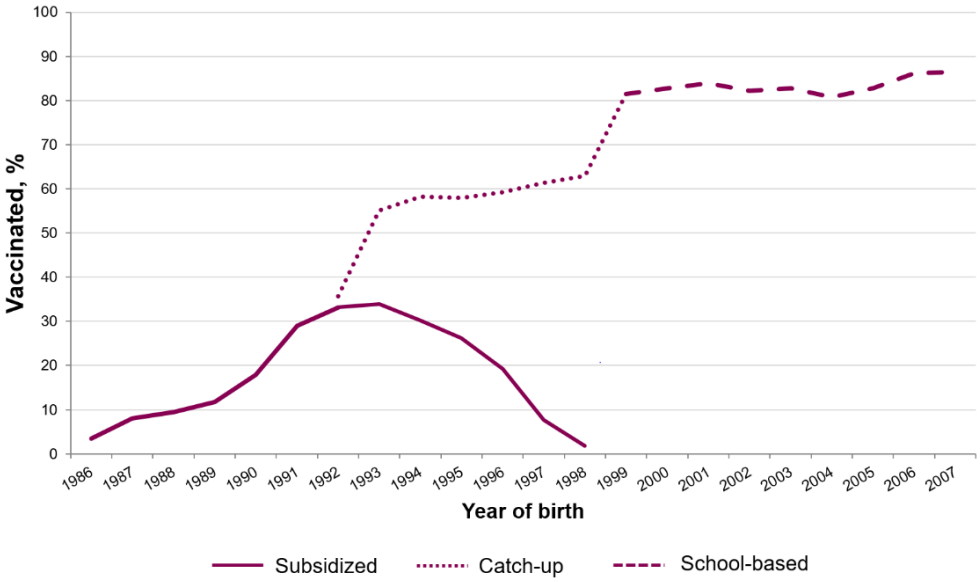

Figure 2.3 *Coverage of at least one dose of HPV vaccine in Sweden, by 2019-12-31.* Provided by Sparén, 2020.

2.2.2.2 *Efficacy, safety and effectiveness of HPV vaccination*

The available prophylactic HPV vaccines have been shown to be remarkably safe and effective. Randomized controlled trials have proven the vaccine efficacy against HPV infection and CIN2+ for the bivalent, quadrivalent, and 9-valent vaccines (9-11). Efficacy on cross-protection against non-vaccine HPV type 31 has been reported for quadrivalent HPV vaccine, and the bivalent vaccine provides further cross-protective efficacy for HPV types 31, 33, 45 and 51 (73). The safety profiles of HPV vaccines have been demonstrated in randomized

controlled trials (74-78). After implementation of HPV vaccination, observational studies also supported that HPV vaccination was not related to increased risk of autoimmune, neurological and venous thromboembolic adverse events (79, 80), multiple sclerosis or other demyelinating diseases (81), or adverse pregnancy outcomes (82).

Based on real-life vaccination programs, HPV vaccination was shown to be related to reduced risk of genital warts (83, 84), which is the first observable HPV-related clinical outcome. Effectiveness against high-grade cervical lesions have also been demonstrated in Sweden (85, 86), Scotland (87), Australia (88) and US (89) by comparing vaccinated women to unvaccinated women, usually with a stronger protection for girls vaccinated at an earlier age. Recently, a global meta-analysis on the population-level impact of HPV vaccination showed very promising results on the protection of HPV vaccination against HPV infections, genital warts and CIN2+ by comparing the post-vaccination period to the pre-vaccination period (17, 90). A herd-immunity effect among female population with vaccine coverage over 50% was also recorded (90). Even though HPV vaccination has been proven to be effective against various HPV-related outcomes, the effectiveness against cervical cancer is very limited.

2.2.2.3 Screening in HPV-vaccinated cohorts

The performance of screening methods can be measured by sensitivity and specificity. In addition to that, the positive predictive value (PPV) and NPV are also important indicators of performance for a diagnostic test, and they are usually affected by the prevalence of diseases (91). According to the Swedish screening guidelines, women below age 30 will continue to be tested with cytology considering the high HPV prevalence among this age group. Since the HPV vaccinated women are well protected against high-grade cervical lesions of cervical cancer, a substantial drop in the prevalence of high-grade cervical lesions is expected when HPV vaccinated birth cohorts enter the screening program (92). Decreased prevalence of cervical lesions from the HPV vaccination program will lower the PPV assuming an unchanged sensitivity (91, 93, 94). The consequential increase of false-positive results might potentially increase the diagnostic tests, psychological stress for women (93) and impact pregnancy outcomes (95) if unnecessary treatment is performed.

In Sweden, birth cohorts vaccinated through opportunistic HPV vaccination and free-of-charge catch-up program, with low or moderate vaccine coverage, have already entered the screening program. When the birth cohorts vaccinated through school-based vaccination program (coverage over 80%) enter the screening program in 2022, this problem will be exacerbated. A heavy drop in the PPV of the screening test will make cytological screening very inefficient (93). As a contrast, HPV testing increases protection against cervical cancer with a 60% -70% decrease of incidence compared to cytology for women aged 30 and above (18). It also gives a higher NPV compared to negative cytology test, potentially allowing extension of screening interval for women with HPV negative results from screening program (96).

In Scotland, the PPV of cytology for CIN2+ was found to be lower (16%) for vaccinated women compared to unvaccinated women (97). Results from the Compass trial in Australian

(98) showed an increased detection of CIN2+ by using HPV primary screening compared to using cytology in HPV-vaccinated birth cohorts, and higher but not statistically different referral rate for colposcopy for HPV-screened compared to cytology-screened women.

2.3 CERVICAL CANCER PROGNOSIS

Cervical cancer accounts for substantial morbidity and mortality in women worldwide. The five-year relative survival of cervical cancer varies across countries, and in Europe, the five-year relative survival for women with cervical cancer was around 62% during 2000–2007 (99). In Nordic countries, the five-year relative survival for cervical cancer ranged from 66% to 70% during 2012-2016, and was 69% for women in Sweden (100).

2.3.1 Clinical and demographic characteristics

The prognosis of cervical cancer has been shown to be associated with several clinical characteristics such as age, cancer stage, and histopathological features (99, 101). Age usually has a negative effect on prognosis (99). The clinical stage of cervical cancer is often determined by the clinical features at the time of diagnosis, and mainly depends on the size of tumor, and risk of spread to the pelvis. It is usually classified according to the International Federation of Gynecology and Obstetrics (FIGO) staging system (102). FIGO stage is a strong indicator for prognosis of cervical cancer (56, 101), and the probability of survival decreases as the stage increases. In Sweden, micro-invasive (Stage IA) represents around 20% of all cases with younger age of cancer diagnosis and a favorable 5-year prognosis (almost 100%). Localized cervical cancer (Stage IB) represents about 40% of all cases and the cure proportion is 85%–90%. The rest 40% cases are advanced (Stages II+), and they tend to have worse 5-year survival (lower than 50%) (56). Grade is also associated with different clinical prognosis (101). The uncommon histological types of cervical cancer showed a poorer prognosis compared to the two major types (56, 101, 103). Other factors including education (104) and tobacco smoking (105) were also suggested to be linked with cervical cancer prognosis.

2.3.2 HPV status and prognosis

Apart from clinical and demographic factors, how HPV in the invasive cervical tumor tissue is associated with prognosis once cancer occur has been investigated, but findings were conflicting (106-112). Most of the previous studies (106-109) have shown that undetectable HPV in tumors was associated with poorer prognosis compared to HPV-positive cervical cancer. However, some studies reported no difference in prognosis in relation to tumor HPV status (110, 111). Recently, a study evaluating the pattern of HPV integration and prognosis (113) showed that the extent to which the HPV was integrated can be an indicator for prognosis. Moreover, specific HPV types might also influence the prognosis of cervical cancer, but the findings were inconclusive (106, 107, 114-116).

3 AIMS AND RESEARCH QUESTIONS

This thesis addresses a range of topics associated with prevention and prognosis of cervical cancer. It aims to provide scientific evidence on effectiveness of the preventive procedures against cervical cancer including HPV vaccination and cervical screening, to support the evidence-based decision-making for future screening strategies in post HPV vaccination era, and to evaluate HPV in cervical tumors as a potential biomarker for the prognosis of invasive cervical cancer.

The specific research questions for each paper are illustrated in Figure 3.1.

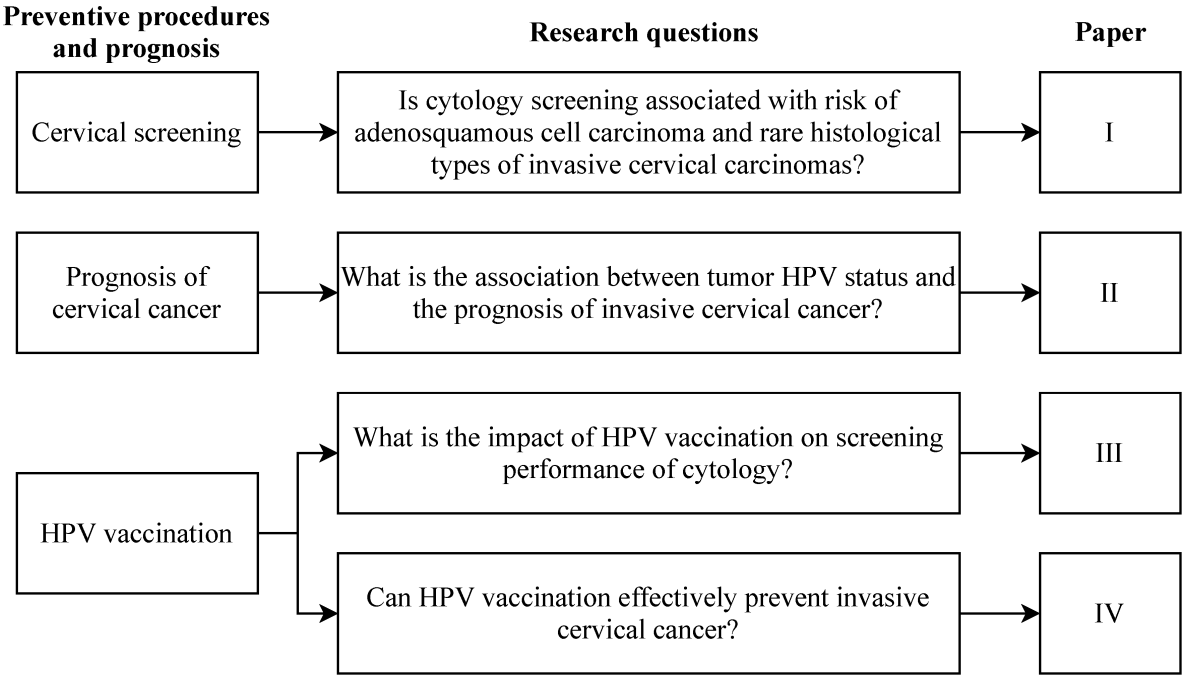

Figure 3.1 *Overview of the thesis and research question for each paper.* HPV, human papillomavirus.

4 DATA SOURCE AND LINKAGE

4.1 ADVANCING CERVICAL CANCER ERADICATION STRATEGIES (ACCES) DATABASE

Data utilized in this project was based on the ACCES database. The ACCES database was established in 2006, with the aim to study cervical cancer prevention with focus on HPV vaccination and cervical screening. The ACCES database compiled information from Swedish National Cervical Screening Registry (NKCx), National Cervical Cancer Audit Database, Swedish population and healthcare registers managed by National Board of Health and Welfare (NBHW, Socialstyrelsen), Statistics Sweden (Statistika Centralbyrån, SCB), and the Public Health Agency of Sweden (Folkhälsomyndigheten) (Figure 4.1). Data were linked at individual level. Up to now, the ACCES database has been updated to year 2017, and contains information for 7.7 million Swedish women.

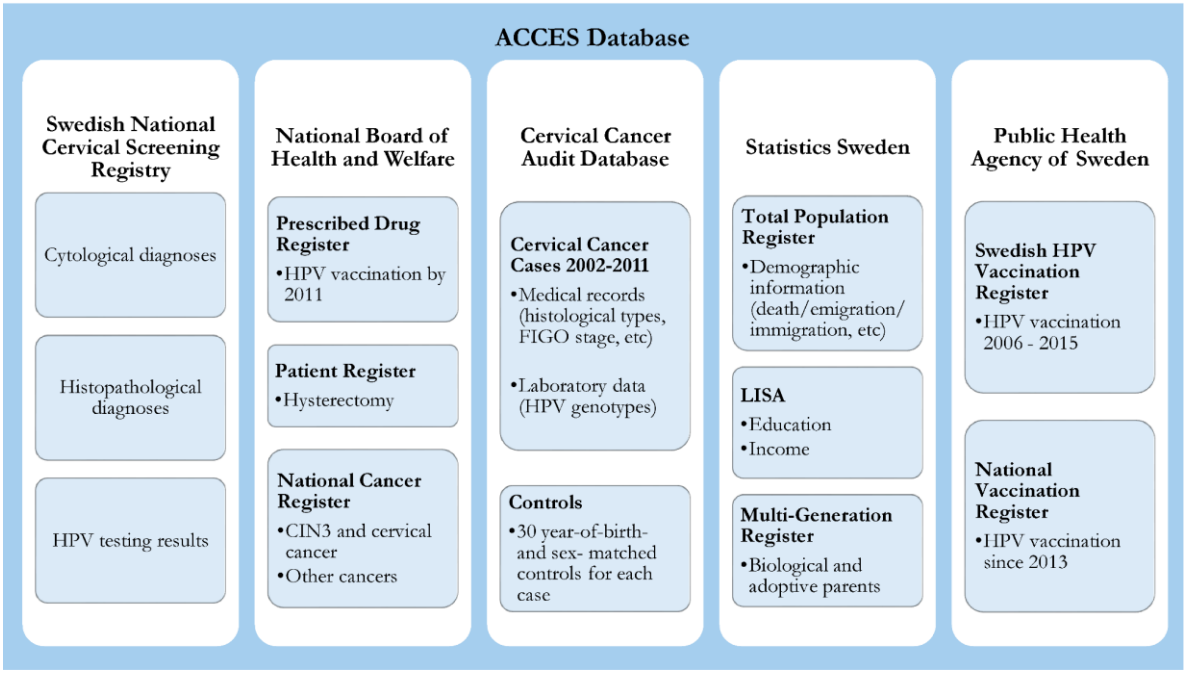

Figure 4.1 *Framework of Advancing Cervical Cancer Eradication Strategies (ACCES) database and information used in the included constituent papers.* HPV, human papillomavirus. CIN3, cervical intraepithelial neoplasia grade 3. FIGO, International Federation of Gynecology and Obstetrics. LISA, Longitudinal integration database for health insurance and labour market studies.

4.1.1 Swedish National Cervical Screening Registry (NKCx)

NKCx is a quality register, which aims to provide evidence base for the prevention of cervical cancer in Sweden. It serves as a base for systematical and regular surveillance and evaluation on cervical screening program in reducing incidence and mortality of cervical cancer, as well as adverse events of preventive interventions (117). NKCx was established in year 2000, and has complete coverage of cervical screening in the Swedish female population since 1995. Laboratories of cytology and histopathology in each county perform the HPV-testing, cytological and histopathological examinations on samples and report to NKCx. HPV-testing

results are also collected from the microbiological laboratories (118). NKCx captures invitations to cervical screening and women's entire screening history, including cytological, HPV-testing and histological diagnoses, both within organized screening program and opportunistic screening tests.

4.1.2 The National Cervical Cancer Audit Database

The National Cervical Cancer Audit Database comprised of 4254 confirmed primary invasive epithelial cervical cancer diagnosed during 2002-2011 and thirty year-of-birth- and sex-matched controls for each case. All cases were reviewed by a senior gynecologist and histo-pathologist to retrieve the medical records and confirm diagnosis. Information on FIGO stage, treatment (including hysterectomy, radiation, chemotherapy and palliative care), and histological classification were available. Formalin-fixed paraffin-embedded (FFPE) tissue diagnostic blocks were collected from biobanks throughout Sweden for all confirmed cervical cancer cases, and 2850/4254 confirmed cases had valid HPV genotypes based on L1-region. Information on HPV16-E7 and HPV18-E6 were available for HPV-L1-negative cases.

4.1.3 Swedish population and healthcare registers

Total Population Register

Individuals residing in Sweden on a permanent basis (planning to live in Sweden for one year or more) were registered in the Total Population Register (TPR) with an assigned Swedish personal identity number (PIN) every end of the year since 31 Dec, 1968 (119). TPR contains an excerpt from the Tax Agency Population Register on a daily basis, and consists of life events including date of birth, gender, country of birth, death, emigration, immigration to Sweden and migration within Sweden, etc. TPR is usually used to identify at risk population controls, study population for cohorts, and calculate follow-up time.

Swedish Cancer Register

Swedish cancer register (SCR), established in year 1958 and maintained by NBHW, is a mandatory reporting register covering the whole Swedish population. Since the mid-1980s, the six regional cancer centers have been responsible for the coding and registration of cancer if the notifications are sent by healthcare providers (120). After verification and correction, it is sent annually to the NBHW to be included in the national cancer register. SCR comprises information of patient data (gender, place of residence at diagnosis), medical data (site of tumor, histological types, and date of diagnosis), and follow-up data (date and cause of death, migration). Information on cancer stage has been systematically recorded since 2004. International Classification of Diseases for Oncology, 3rd Edition (ICD-O-3) and International Classification of Diseases (ICD revisions 7-10) were used for the registration of site and type of tumor. The WHO C24 histopathological code has been used for histological types classification since 1958. The underreporting of SCR is very low and without major impact for research and surveillance purpose.

The Swedish Multi-Generation register

The Swedish Multi-Generation register (MGR) is part of registration of TPR in SCB. It includes population (index person) born since 1932 onwards and alive by 1961. The register collects information on parental records of the index person, with information available on mothers for 97% and on fathers for 95% of the index persons (121).

Prescribed Drug Register

The Prescribed Drug Register (PDR) was established in July 2005 and is maintained by the NBHW (122). It contains information on prescribed drug utilization and expenditures with complete coverage since it was established. All drug prescriptions are automatically registered using Anatomical Therapeutic Chemical (ATC) codes, including vaccine prescriptions which are relevant to this thesis. Inpatient prescriptions and over-the-counter drugs are not registered in PDR.

Swedish HPV Vaccination Register

Swedish HPV Vaccination Register (SVEVAC), initiated by Public Health Agency of Sweden in year 2002, was a nationwide registration of HPV vaccination between 2006 and 2015. It is a voluntary reporting register requiring inform consent from the individual or their parent. Vaccination is registered anonymously if no inform consent is available. This corresponds to a registration record without PIN, but information on sex, birth year and date of vaccination are registered. In 2012, the anonymous vaccinations in SVEVAC increased in certain counties due to the change from opt-out to opt-in informed consent as well as online registration platform. It was estimated that around 85% - 90% of completed HPV vaccinations were reported in SVEVAC according to the number of vaccine doses sold (123). Around 25% of the vaccinations are registered anonymously in SVEVAC.

National Vaccination Register

The Public Health Agency of Sweden is responsible for the National Vaccination Register (NVR) (124). Since January 2013, all healthcare professionals were obligated to report vaccinations administered within the childhood vaccination program to NVR. Information in NVR covers date of the vaccination, PIN, vaccine used, batch number and the healthcare provider responsible for the vaccination. HPV vaccination through the school-based program has been registered in NVR with identity since 2013. HPV vaccinations taken within the vaccination program from birth cohort 1999 onwards and up to age 21 are recorded in NVR.

Patient register

The patient register (PR) was established in 1964 and has achieved nationwide coverage since 1987. The register comprises information on hospital discharge, diagnosis and surgical procedures. Swedish international classification system was used to register the diagnosis (1964-1968: ICD-7, 1969-1986: ICD-8, 1987-1996: ICD-9, and 1997 onwards: ICD-10). The

register contains patient information on all doctor visits in closed and specialized open health care. Since 2001, out-patient hospital visits from both public and private care providers have also been registered in the PR. The overall coverage of PR is around 80% (almost 100% for public hospital visits) (125). Data from primary care is not in the register. Since 2015, the reporting to the PR has been performed monthly instead of annually.

Longitudinal integration database for health insurance and labour market studies

The longitudinal integration database for health insurance and labour market studies (LISA) database is operated by SCB, and included individuals aged 16 and above that were registered in Sweden during 1990-2009, and individuals aged 15 and above since 2010 (126). The database integrates data from the educational sectors, social sectors and labor market, and updates annually with a separated new register. Information in LISA database includes education, individual income and family income.

4.2 ETHICAL CONSIDERATIONS AND DATA LINKAGE

The studies included in this thesis are largely depended on data from Swedish population and healthcare registries. In Sweden, the register-based research is protected by law, and all projects should be approved by the Ethical Review Authority (former Ethical Review Boards). Register-based research is commonly not deviated from clinical routine nor involved direct contact with the study participants, and therefore, inform consent could be waived.

Once the ethical permit for the project is granted, data is sent to SCB and linked based on the Swedish PIN, which was established in 1947 and serves as a unique identifier in Swedish healthcare system as well as many other areas of the society (127). The PIN enable linkages between population registers and large-scale medical registers allowing for virtually 100% complete coverage in Swedish healthcare system. The SCB replaces the PIN by a corresponding sequential number before data is delivered for research purposes. Data usage is under strict regulation, and delivered data can not be used for purposes other than approval from the Ethical Review Authority. Re-use of data is possible if the analysis is within the field covered by approval, or an amendment of ethical approval for that specific research purpose is granted.

All studies included in this thesis were approved by the Regional Ethical Review Board in Stockholm. Informed consent from the study participants was waived due to the population-based nature of the study. Ethical approvals related to this thesis work include: Dnr 2011/1026-31/4; Dnr 2012/1028/32; Dnr 02-556; Dnr 2011/921-32; Dnr 2012/216-32; Dnr 2012/1426-32 and Dnr 2014/246-32.

5 METHODS

An overview of study design, study population, main measurements and statistical methods for each paper included in the thesis is presented in Figure 5.1.

Paper	Study design	Study population	Exposure & Outcome	Statistical analysis
I	Nested case-control study	Women diagnosed as adenosquamous cell carcinoma and rare histological types of invasive cervical carcinoma during 2002-2011 and their corresponding controls	Exposure: cervical screening Outcome: invasive cervical carcinoma	Conditional logistic regression
II	Population-based cohort study	Women diagnosed as primary invasive epithelial cervical cancer during 2002-2011 and had valid HPV genotypes	Exposure: tumor high-risk HPV status Outcome: all-cause mortality	Relative survival; Poisson regression
III	Population-based cohort study	Women born 1989-1993, resident in Sweden and attend cervical screening at age 23	Exposure: HPV vaccination Outcome: histopathological confirmed CIN2+	Positive predictive values; Log-binominal regression
IV	Population-based cohort study	Female age 15-30 and resident in Sweden during Jan 1, 2006 to Dec 31, 2017	Exposure: HPV vaccination Outcome: invasive cervical cancer	Poisson regression

Figure 5.1 *Overview of methodologies for papers included in thesis.* HPV, human papillomavirus; CIN2+, cervical intraepithelial neoplasia grade 2 or worse

5.1 STUDY DESIGN AND STUDY POPULATION

5.1.1 Nested case-control design

The nested case-control design, which is an extension of the general case-control design, has been widely used in epidemiological research. In a nested case-control study, cases occurring within the defined cohort are identified, and a specific number of controls are selected among population who have not yet developed the defined disease at the time of occurrence of an index case (128). The selection of cases and controls are incidence density sampling, also called "risk-set sampling" (Figure 5.2), which allows for matching on time. Matching on other factors (e.g. age and sex) can also be applied in such a design to rule out a potential confounding effect. A nested case-control design was used in paper I.

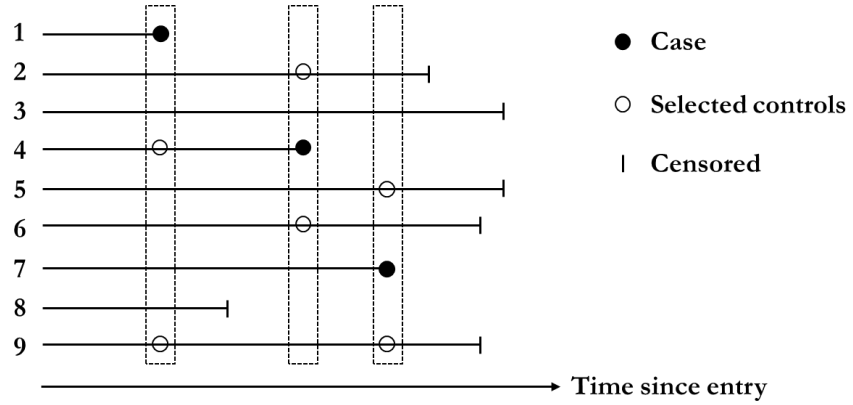

Figure 5.2 *Illustration of incidence density sampling in nested case-control design.* The sampling ratio of cases to controls here is 1:2.

5.1.1.1 Paper I

We aimed to examine the association between cervical cytology screening and risk of ASC and rare histological types of invasive cervical carcinoma (RICC). Cases and controls were identified from a cohort of Swedish women born between 1909-1986 with a follow-up during 2002-2011. We included women diagnosed as ASC and RICC (n=338) and thirty year-of-birth-matched controls for each case, randomly selected from TPR utilizing incidence density sampling. All controls were alive, with no history of cervical cancer and living in Sweden before the cancer diagnosis of their corresponding case. Among those, 449 controls who had total hysterectomy were excluded, because they were not at risk of cervical cancer from that point in time. In total, we included 9691 matched controls.

5.1.2 Population-based cohort design

Cohort studies track time from exposure to outcome between at least two groups of individuals either prospectively or retrospectively, and are usually helpful in investigating the incidence or natural history of diseases (129). Cohort studies require clear and objective definitions of both exposure and outcome, and are commonly used for examining multiple outcomes after a single

exposure. In such a design, all participants included (both exposed and unexposed groups) should be at risk of developing the diseases of interest, and importantly the unexposed group should resemble the exposed group in all aspects except for the absence of exposure. The population-based cohort design was used for papers II, III & IV.

5.1.2.1 Paper II

We aimed to evaluate the association between tumor HPV status and invasive cervical cancer prognosis. We included invasive cervical cancer cases diagnosed in Sweden during 2002-2011 (confirmed as primary invasive epithelial cervical cancer), and with valid HPV results based on the archived diagnostic blocks (Figure 5.3). A total of 2845 cases were included and prospectively followed up from date of cancer diagnosis to 31 December, 2015, migration from Sweden, or death, whichever occurred first.

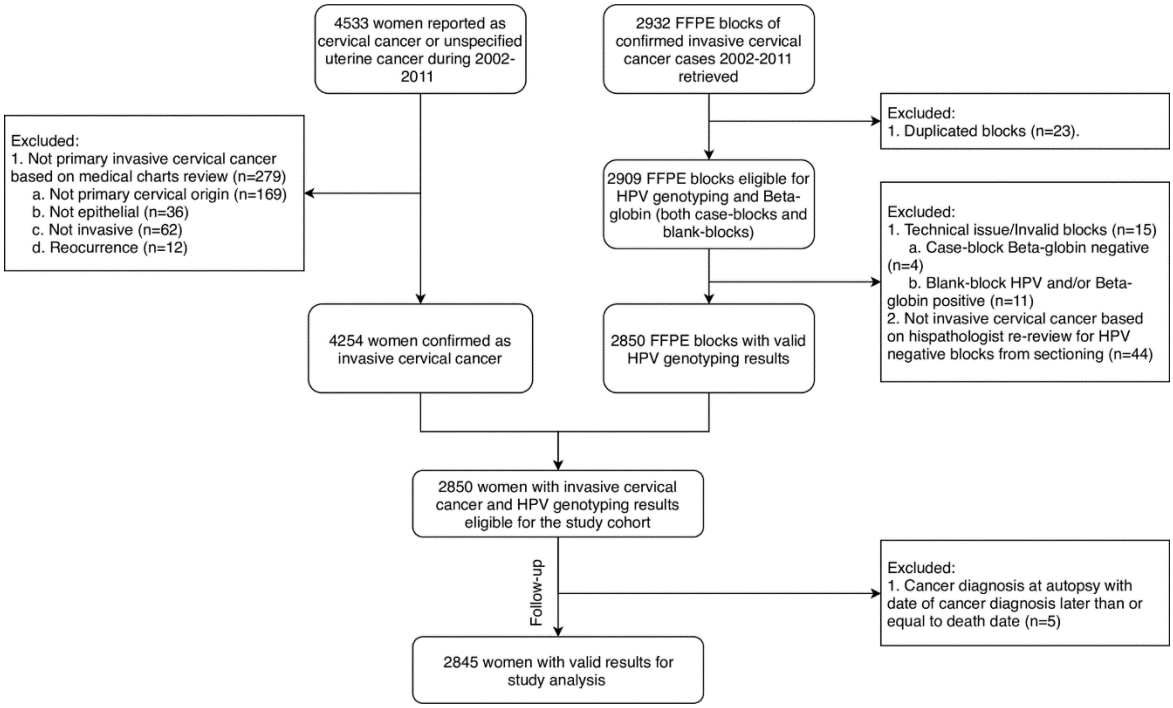

Figure 5.3 *Study population for paper II.* HPV, human papillomavirus; FFPE, formalin-fixed paraffin-embedded.

5.1.2.2 Paper III

The aim was to examine how HPV vaccination would affect the PPV of cytology. We identified women born between 1989 and 1993, who were resident in Sweden since the introduction of HPV vaccination (October, 2006) and eligible for screening. Among those, we included women who attended cervical screening at age 23 and had valid histopathological diagnosis based on records from NKCx (Figure 5.4).

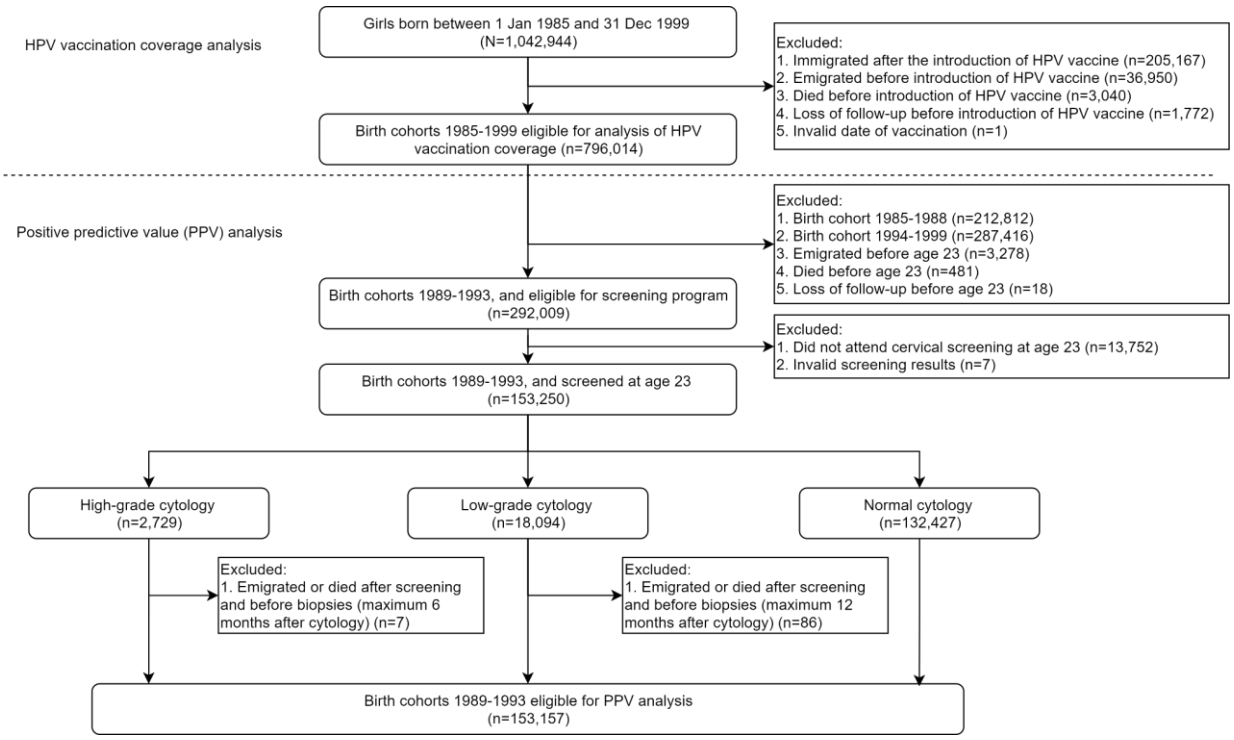

Figure 5.4 *Study population for paper III.* HPV, human papillomavirus.

5.1.2.3 Paper IV

To investigate the association between HPV vaccination and risk of invasive cervical cancer, we included a cohort of women age 15 to 30 during 2006-2017 (Figure 5.5). The entry of the study was defined as either age 15 or 1 Jan, 2006 whichever came last. We excluded women who immigrated to Sweden after 1 Jan, 2006, died, emigrated or were lost to follow-up before entry of the study, and women with a diagnosis of invasive cervical cancer before entry to the cohort. We followed all eligible participants prospectively to a diagnosis of invasive cervical cancer, death, emigration, 31st birthday or 31 December, 2017 whichever came first.

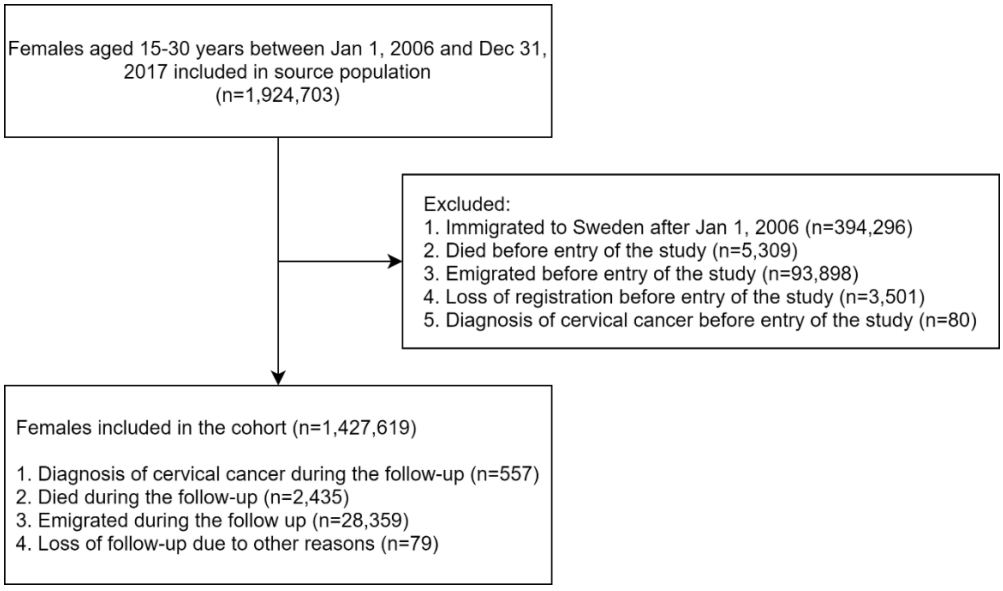

Figure 5.5 *Study population for paper IV.*

5.2 MAIN MEASUREMENTS

5.2.1 Invasive cervical cancer (papers I, II & IV)

Invasive cervical cancer in papers I and II were based on the National Cervical Cancer Audit Database (derived from SCR), where all cases were confirmed as primary, invasive, epithelial cancer with cervix origin after review of medical records and histopathological review. The histological types were classified as squamous cell carcinoma, adenocarcinoma, ASC and RICC (including glassy cell carcinoma, clear cell carcinoma, small cell carcinoma, neuroendocrine carcinoma, large cell carcinoma, and undifferentiated carcinoma). Cancer stage was classified based on the FIGO staging system, and categorized as stage IA (microinvasive), IB (localized), and II+ (advanced cancer) (102). In paper IV, the invasive cervical cancers were retrieved from SCR using ICD-10 (code: C53).

5.2.2 Cervical screening (papers I & III)

Cervical screening was obtained from NKCx. In paper I, cervical screening was measured during the last two recommended screening intervals 6 months prior to diagnosis of cervical cancer, with regard to age at cancer diagnosis (Figure 5.6). We measured the cervical screening as screening status and screening history. Screening status was classified as "no test", "one test", and "two tests". Screening history was categorized as "no test", "double normal results", "one normal result only" and "at least one abnormal result" based on the cytological results. The cytological and histopathological diagnoses were based on the Systematized Nomenclature of Medicine (SNOMED) codes defined by the Swedish Association of Clinical Cytology and Swedish Society of Pathology, respectively (Table 5.1).

In paper III, cervical screening was measured as both cytological results and histopathological diagnoses. Women's first cytology at age 23 were classified as normal cytology, low-grade cytology and high-grade cytology (Table 5.1). If an abnormal cytological result was present, we further linked to histopathological diagnosis and identified women confirmed as CIN2+ within 6 months after high-grade cytology, and within 12 months after low-grade cytology.

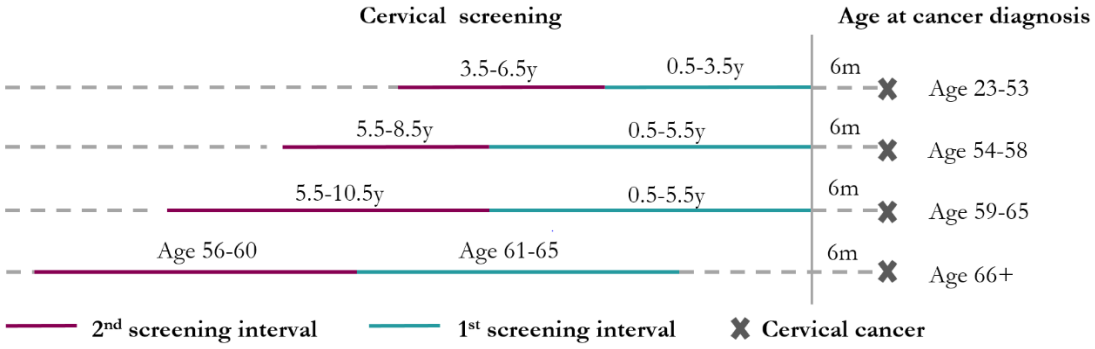

Figure 5.6 *Evaluated screening intervals depending on age at cancer diagnosis.* Screening tests taken within 6 months prior to diagnosis of cervical cancer was disregarded as they were diagnostic tests and not preventing cervical cancer.

Table 5.1 *Systematized Nomenclature of Medicine (SNOMED) codes for cytological and histopathological diagnoses.*

Outcome	SNOMED code
Normal cytological diagnoses	
Benign sample	M00110
Abnormal cytological diagnoses	
Low-grade cytology	
Atypical squamous cell of undetermined significance (ASCUS)	M69710
Mild dysplasia/Cervical intraepithelial neoplasia (CIN) 1	M74006
High-grade cytology	
Atypia in cells of uncertain origin	M69700
Atypical glandular cells (AGC)	M69720
Moderate dysplasia/Cervical intraepithelial neoplasia (CIN) 2	M74007
Severe dysplasia/Cervical intraepithelial neoplasia (CIN) 3/Cancer in situ (CIS)	M80702
Suspected high-grade dysplasia (ASC-H)	M69719
Squamous cell carcinoma	M80703
Adenocarcinoma/Adenocarcinoma in situ (AIS)	M81403
Histopathological diagnoses of CIN2+	
Cervical intraepithelial neoplasia grade 2	M74007
Cervical intraepithelial neoplasia grade 3	M80702
Carcinoma in situ	M80762
Adenocarcinoma in situ	M81402
Adenosquamous cell cancer in situ	M85602
Invasive cervical cancer of any origin	M80703, M81401, M81403, M85601, M85603, M80413

5.2.3 HPV genotypes (paper II)

HPV genotypes were derived from the National Cervical Cancer Audit Database. FFPE diagnostic samples for all confirmed invasive cervical cancer cases during 2002-2011 were collected from biobanks in Sweden. The available FFPE blocks were extracted and tested in parallel with β-globin real-time polymerase chain reaction (PCR). We performed the HPV typing using general primers (MGP)-PCR targeting the L1 region (130), followed by typing with Luminex for 13 high risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and non-high risk types (6, 11, 26, 30, 40, 42, 43, 53, 54, 61, 66, 67, 69, 70, 73, 74, 81, 82, 83, 86, 87, 89, 90, 91) (28, 131). Real-time PCR test for HPV16-E7 and HPV18-E6 was performed for samples turned out to be HPV-negative based on L1 region (132). This was because the L1 region might lost during the carcinogenesis, while the oncogenic E6 and E7 regions were more likely to be retained (133). To investigate the distribution of HPV genotypes in tumor tissues for ASC and RICC in paper I, the tumor HPV genotypes were categorized as HPV 16, HPV18, other high-risk human papillomavirus (hrHPV)-positive and hrHPV-negative. In paper II, tumor HPV status was the exposure, and classified as hrHPV-positive and hrHPV-negative based on the L1 region.

5.2.4 HPV vaccination (papers III & IV)

Records of HPV vaccination in paper III and IV were retrieved from three different registers including PDR, SVEVAC, and NVR. As SVEVAC had issues with anonymized registration of vaccination, the vaccination records were further complemented with dispensation dates of HPV vaccines from PDR based on ATC codes. The procedure was done following an algorithm

that HPV vaccine dispensed at least 14 days prior to the vaccination administration date were considered as a new dose. In both papers, vaccinated women were defined as vaccinated with at least one dose of HPV vaccination. Otherwise, women were classified as unvaccinated. HPV vaccination was also measured as age at vaccination initiation, and the cut-off of age at vaccination initiation was based on the previous studies on effectiveness of HPV vaccination (84-86).

When we studied the effectiveness of HPV vaccination in paper IV, HPV vaccination was treated in a time-varying manner based on the first dose of HPV vaccination (Figure 5.7). Namely, if the vaccination occurred before study entry, the individual contributed person-time to the vaccinated group since entry to the study. If the vaccination occurred during the follow-up, the individual started to contribute person-time to the unvaccinated group, and switched to the vaccinated group from the date of vaccination; otherwise they remained in the unvaccinated group throughout the study period.

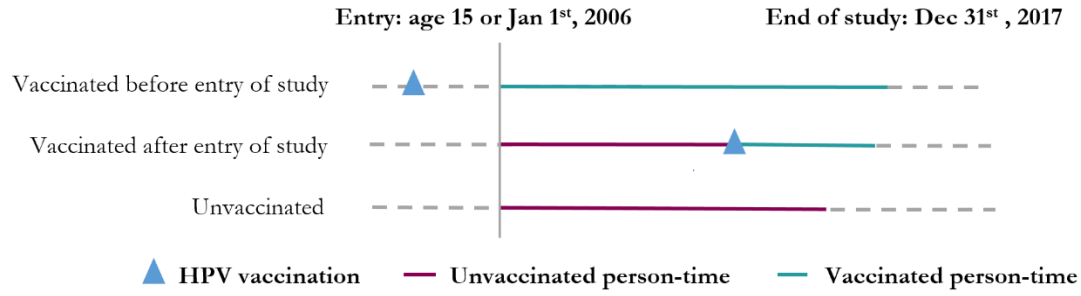

Figure 5.7 *Illustration of time-varying exposure of HPV vaccination in paper IV.* HPV, human papillomavirus.

5.2.5 Covariates

Several covariates were retrieved from national healthcare registers and used as exclusion criteria for study population or adjusted as potential confounders. In the control selection of paper I, we excluded controls who had a total hysterectomy before the index date based on information from PR, because they were no longer at risk of having invasive cervical cancer after such a procedure. We also measured socioeconomic status as education and income, which we retrieved from LISA. The educational level was classified as low (less than high school), middle (high school), high (university and above) and missing, and controlled in statistical analysis in paper I, II & IV. Income level was used in paper IV, and categorized as low, middle, high and missing based on tertiles of income level among the population aged 35-65 during years 2006-2016. Missing values of both educational level and income level were treated as separated subgroups, and included in the regression models. Besides, disease history of study participants' mother on previous diagnosis of cervical intraepithelial neoplasia grade 3 or worse (CIN3+) or any types of cancer were obtained from SCR, and controlled for as confounders in paper IV.

5.3 STATISTICAL METHODS

5.3.1 Conditional logistic regression

Logistic regression investigates the association between a dichotomous dependent variable and explanatory variables using a logit model. Conditional logistic regression is a specialized type of logistic regression that also accounts for matching and stratification. The likelihood of the conditional logistic regression model is formulated so that cases and controls are only compared within the same riskset, by assuming constant odds ratios (ORs) across matched strata, and controlling for the matched factors through conditioning.

In paper I, we estimated the ORs of ASC and RICC in relation to screening status and screening history, together with 95% confidence intervals (CIs), based on conditional logistic regression, further adjusted for the highest educational level achieved by the year of cancer diagnosis. These ORs were interpreted as incidence rate ratios (IRRs) (134, 135).

5.3.2 Relative survival

Relative survival is calculated as the all-cause survival of a cancer population, divided by the expected all-cause survival of that population if they were cancer-free. Relative survival can be used as an estimate for net survival, which measures the survival probability after a cancer diagnosis while removing deaths due to other causes. Crucially, net survival does not depend on the availability of reliable cause of death information. The corresponding concept to relative survival on the hazard scale is named excess mortality, defined as the difference between the all-cause mortality observed in the cohort of cancer population and the expected mortality if the population were cancer-free (136). Relative survival relies on two general assumptions: firstly, that the expected mortality in the cancer population and the general population are independent, conditional on the matching variables (e.g. age, sex, calendar year) and covariates included in the model; secondly, that the expected mortality in the general population is exchangeable with the mortality in the cancer population if they were cancer-free.

In paper II, we estimated net survival within the relative survival framework, assuming that cervical cancer cases were comparable to the general female population of the same age during the same calendar period if they did not have cancer. The life table method (136) was used to estimate five-year relative survival ratios (RSRs) in relation to tumor hrHPV status.

5.3.3 Poisson regression

The Poisson regression model is a type of generalized linear model, which is commonly used for count data, but can also be applied to time-to-event data (137). In survival analysis, a Poisson regression models the incidence rates (IRs), and provides estimates for the log-IRRs relative to the baseline IR for each comparison group via their regression coefficients. The shape of the baseline IR can be chosen to fit the data, from a simple constant rate across the follow-up time, piece-wise constant rate over shorter intervals of follow-up time (i.e. a step function), or as a restricted cubic spline smooth over the same shorter time intervals. The

Poisson model is a parametric model, generally assuming proportional hazards, though it can model non-proportional effects via interactions with the time scale.

In paper II, we used a Poisson regression model to estimate both crude and adjusted excess hazard ratios (EHRs) with 95% CIs, assuming piece-wise constant hazards in in 1-year time bands after diagnosis; we also adjusted the model for age at diagnosis, FIGO stage, time after cancer diagnosis, and educational level. In paper IV, we used Poisson regression to model the IR of cervical cancer over attained age in 1-year time bands up to age 30 using a spline term, and estimated the IRRs (with 95% CIs) between vaccinated and unvaccinated women, controlling for county of residence and parental characteristics.

5.3.4 Log-binominal regression

Log-binominal regression is a generalized linear model in the binomial family, with a log link function, and commonly used as an alternative to the logistic regression model for cohort data where the outcome is not rare. The coefficients from log-binomial regression model can be interpreted as estimated risk ratios (RRs). In paper III, the PPV is calculated as the number of women screen-positive and confirmed as CIN2+ in histopathological diagnosis, divided by the number of women screen-positive. To investigate changes in PPV, we modelled the crude and birth-cohort-adjusted RRs of PPV for CIN2+ with 95% CI in relation to age of HPV vaccination initiation, using log-binominal regression. The change of PPV was presented as (1-RR)*100%.

6 MAIN FINDINGS

6.1 CERVICAL SCREENING AND RISK OF ADENOSQUAMOUS CELL CARCINOMA AND RARE INVASIVE CERVICAL CARCINOMA.

We included a total of 338 cases, which consist of 164 (49%) cases of ASC and 174 (51%) cases of RICC. The RICC consists of 43 cases of glassy cell carcinoma, 31 cases of clear cell carcinoma, and 100 cases of other rare types of invasive cervical carcinoma.

A reduced risk of ASC (IRR 0.22, 95% CI 0.14 - 0.34) and RICC (0.34, 95% CI 0.21 - 0.55) was observed for women who had screening tests in both of the previous two recommended screening intervals compared to women without any screening tests (Figure 6.1). The risk reduction for women who only had a screening test in one of the recommended screening intervals was attenuated compared to women had screening tests in both intervals (ASC: 0.39, 95% CI 0.26 - 0.59; RICC: 0.69, 95% CI 0.45 - 1.06).

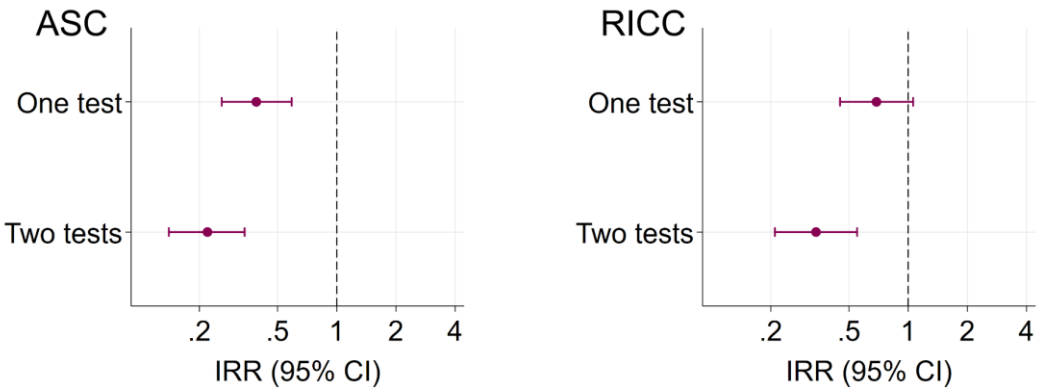

Figure 6.1 *Incidence rate ratio (IRR) of adenosquamous cell carcinoma (ASC) and rare types of invasive cervical carcinoma (RICC) by screening status in the two previous screening intervals in women aged over 30.* Reference group: no test. RICC includes glassy cell carcinoma, clear cell carcinoma, and other rare types. IRR is adjusted for educational level and age. Figures were based on estimates from table 2 in paper I.

With regard to the screening history, women having two normal tests were associated with a significantly lower risk of ASC (0.16, 95% CI 0.10 - 0.26) and RICC (0.29, 95% CI 0.17 - 0.48), respectively. However, having at least one abnormal test was related to an elevated but not statistically significant risk of both ASC and RICC compared to women did not have any tests (Figure 6.2).

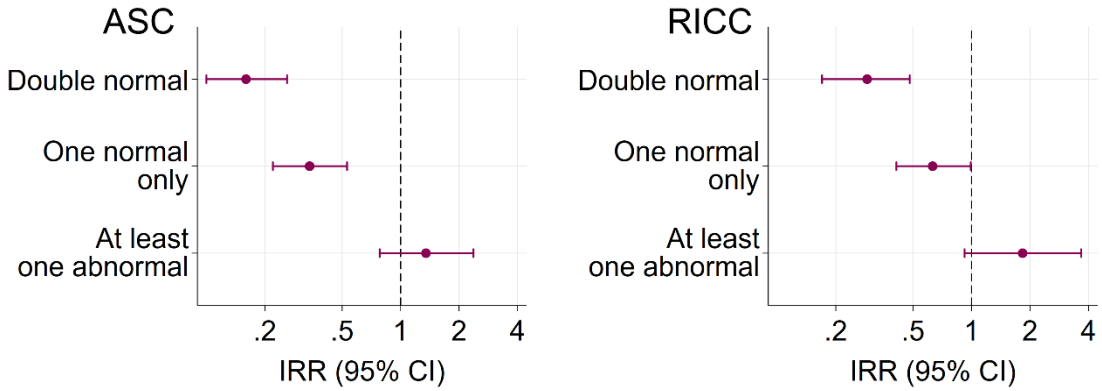

Figure 6.2 *Incidence rate ratio (IRR) of adenosquamous cell carcinoma (ASC) and rare types of invasive cervical carcinoma (RICC) by screening history in the two previous screening intervals in women aged over 30.* Reference group: no test. RICC includes glassy cell carcinoma, clear cell carcinoma, and other rare types. IRR is adjusted for educational level and age. Figures were based on estimates from table 3 in paper I.

Examining the tumor HPV genotypes distribution among 211 cases of ASC and RICC with valid HPV results, we found 148 (70%) cases were hrHPV-positive. HPV18 (37%) was the most common HPV type detected in tumor tissues, and HPV16 and other hrHPV types was detected in 22% and 10% of the cases, respectively (Table 6.1).

Table 6.1 *Tumor human papillomavirus (HPV) status of adenosquamous cell carcinoma and rare types of invasive cervical carcinoma.* Values are number (percentages). Table 5 in paper I.

		Tumour HPV status			
Characteristics	Total	HPV16	HPV18	Other hrHPV positive	hrHPV negative
Adenosquamous cell carcinoma	119 (100)	25 (21)	49 (41)	14 (12)	31 (26)
Rare types of invasive cervical carcinoma:					
Glassy cell carcinoma	24 (100)	5 (21)	12 (50)	3 (13)	4 (17)
Clear cell carcinoma	14 (100)	1 (7)	5 (36)	1 (7)	7 (50)
Other rare types*	54 (100)	16 (30)	13 (24)	4 (7)	21 (39)
Total	211 (100)	47 (22)	79 (37)	22 (10)	63 (30)

Women with valid tumour HPV genotypes were included (n=211).
hrHPV=high risk human papillomavirus.
*Includes small cell carcinoma, large cell carcinoma, neuroendocrine carcinoma, and undifferentiated cell carcinoma.

6.2 TUMOR HIGH-RISK HPV STATUS AND PROGNOSIS OF INVASIVE CERVICAL CANCER.

We included 2845 cases, and the L1 region of hrHPV was found in tumors among 2293 (83.1%) cases. During the follow-up (an average of 6.2 years), we observed 822 deaths (36.3%) in hrHPV L1-positive cases and 309 deaths (56.0%) in hrHPV L1-negative cases.

The hrHPV-positive cases had a constantly better cumulative relative survival than hrHPV-negative cases during the follow-up period (Figure 6.3). The five-year RSR was 0.74 (95% CI, 0.72 - 0.76) for hrHPV-positive cases, 0.54 (95% CI, 0.50 - 0.59) for hrHPV-negative cases. Compared to hrHPV-negative cases, the crude EHR was 0.45 (95% CI 0.38 - 0.52) and the adjusted-EHR was 0.61 (95% CI 0.52 - 0.71) for hrHPV-positive cases. This indicated a 39% significantly lower excess mortality for cases with hrHPV-positive tumors compared to cases

with hrHPV-negative tumors, after controlling for age at diagnosis, FIGO stage, time after cancer diagnosis in 1-year time bands, and educational level.

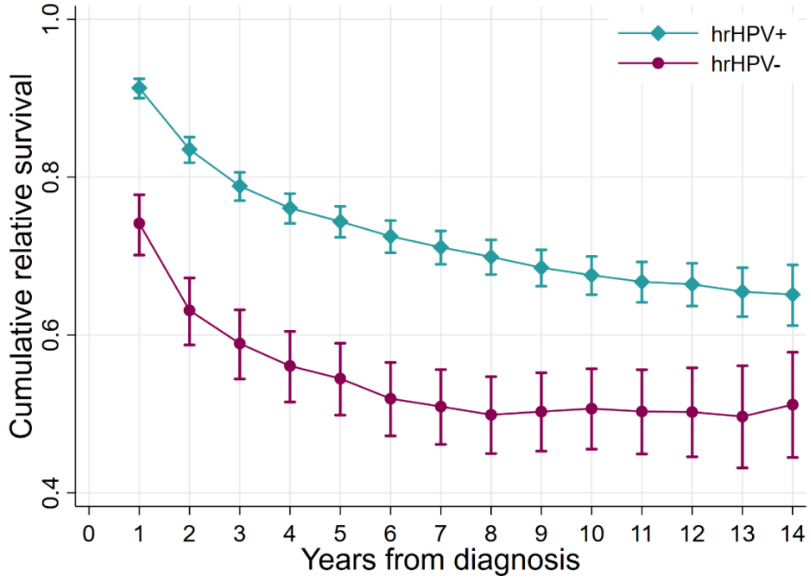

Figure 6.3 *Cumulative relative survival of invasive cervical cancer cases by tumor high-risk human papillomavirus (hrHPV) status.* hrHPV, high-risk human papillomavirus. Fig. 2 in paper II.

Stratified by age at cancer diagnosis, the five-year adjusted EHRs for hrHPV-positive cases were between 0.55 and 0.66 across age groups compared to hrHPV-negative cases (Figure 6.4). After stratifying by FIGO stage, the adjusted EHRs of hrHPV-positive cases were between 0.59 and 0.62 for cases diagnosed at stages above IA compared to hrHPV-negative cases.

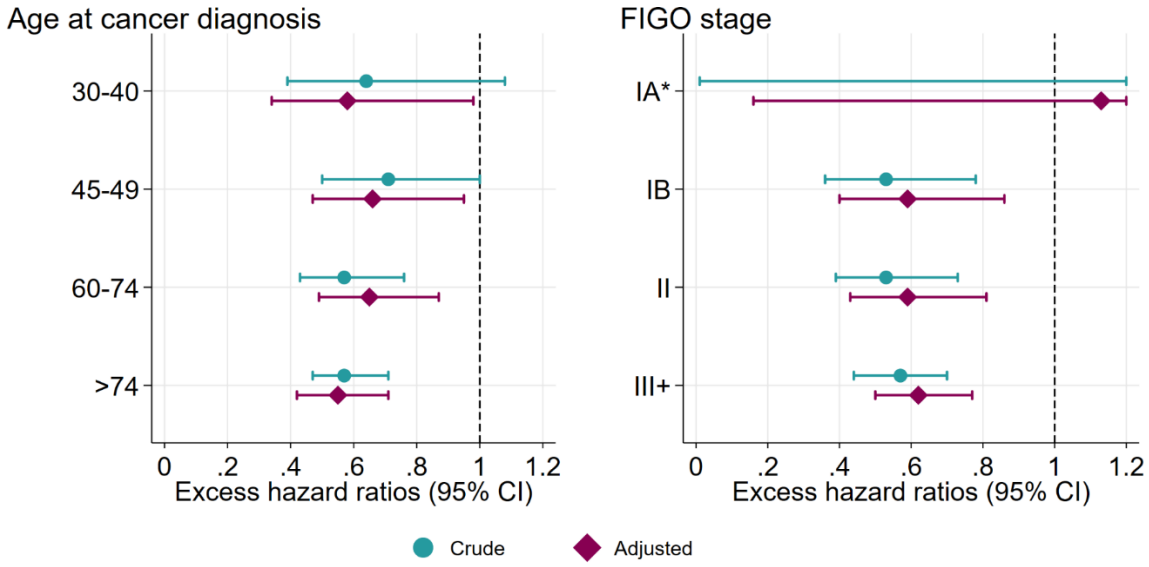

Figure 6.4 *Excess hazard mortality of invasive cervical cancer cases in relation to tumor high-risk human papillomavirus (hrHPV) status by age at cancer diagnosis (left) and FIGO stage (right).* *Upper bound of confidence intervals for cases diagnosed at stage IA were truncated for display purpose. FIGO, International Federation of Gynecology and Obstetrics. Adapted from fig. 3 in paper II.

6.3 SCREENING PERFORMANCE OF CYTOLOGY TEST AMONG HPV-VACCINATED COHORTS.

A total of 153,250 women born during 1989-1993 who attended cervical screening at age 23 were included. Among those, 2,729 (1.8%) women had high-grade cytology, 18,094 (11.8%) women had low-grade cytology, and the rest of 132,427 (86.4%) women had normal cytology.

For women with high-grade cytology, the detection rate of CIN2+ was 1.5% (95% CI, 1.4% - 1.5%), 0.9% (95% CI, 0.8% - 1.0%), and 0.5% (95% CI, 0.5% - 0.6%) for women unvaccinated, initiating vaccination at age 17-22, and initiating vaccination before age 17 (Figure 6.5, Panel A). Correspondingly, the PPV of high-grade cytology for CIN2+ was 69.9% (95% CI, 67.9% - 71.9%), 64.9% (95% CI, 59.8% - 69.8%) and 57.4% (95% CI, 50.9% - 63.7%) (Figure 6.5, Panel C). This corresponded to a PPV reduction of 8% (RR 0.92, 95% CI 0.85 - 1.00) and 17% (RR 0.83, 95% CI 0.74 - 0.93) respectively among women initiating vaccination at age 17-22 and women initiating vaccination before age 17 compared to unvaccinated women, after adjustment for birth cohort.

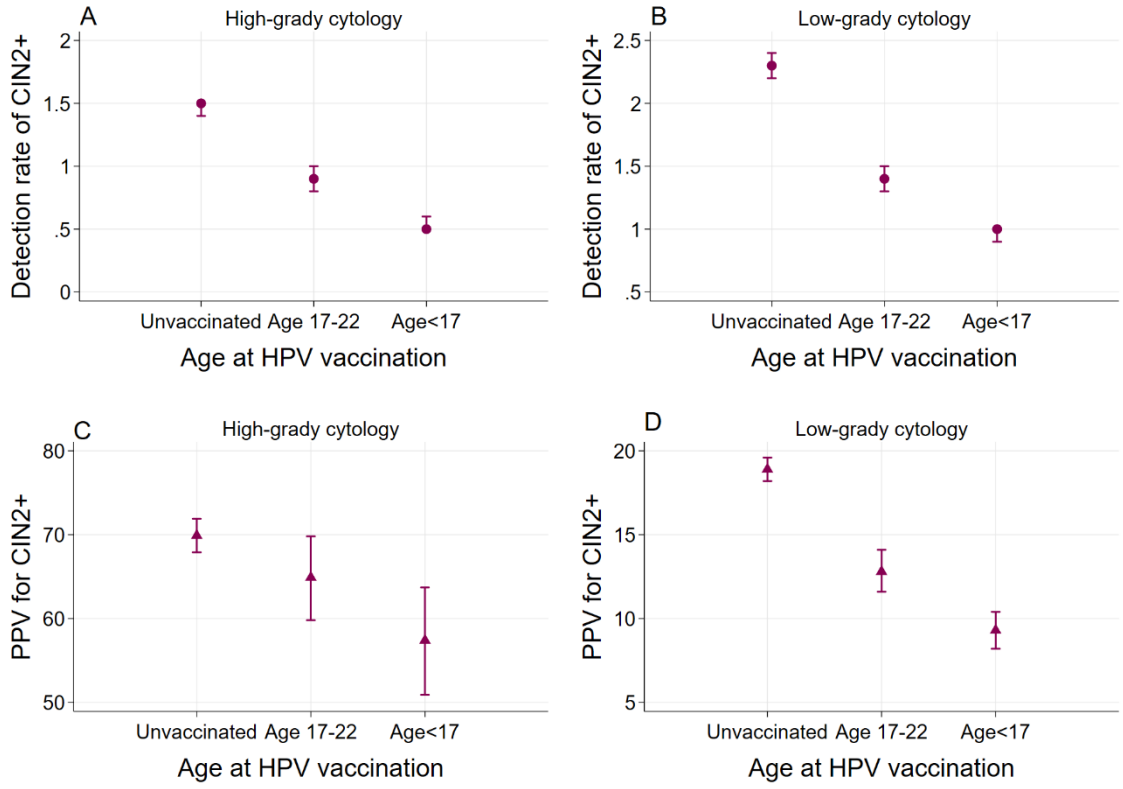

Figure 6.5 *Detection rate (Panel A&B) and positive predictive values (Panel C&D) by age at HPV vaccination.* CIN2+, cervical intraepithelial neoplasia grade 2 or worse. PPV, positive predictive value. Values are percentage. Figures were based on estimates from table 1 in paper III.

Complementary, the detection rate of CIN2+ after having low-grade cytology was 2.3% (95% CI, 2.2% - 2.4%) for unvaccinated women, 1.4% (95% CI, 1.3% - 1.5%) for women initiated vaccination at age 17-22 and 1.0% (95% CI, 0.9% - 1.0%) of women initiating vaccination before age 17 (Figure 6.5, Panel B). The corresponding PPVs for CIN2+ were 18.9% (95% CI,

18.2% - 19.6%), 12.8% (95% CI, 11.6% - 14.1%), and 9.3% (95% CI, 8.2% - 10.4%), respectively (Figure 6.5, Panel D). Compared to unvaccinated women, the PPV declined for women initiating vaccination at age 17-22 (RR 0.72, 95% CI 0.65 - 0.80) and initiation vaccination before age 17 (RR 0.56, 95% CI 0.49 - 0.63) after adjustment for birth cohort.

6.4 HPV VACCINATION AND RISK OF INVASIVE CERVICAL CANCER.

A cohort of 1,427,619 women aged 15-30 during 2006-2017 were included, and 383,694 (26.9%) of them were vaccinated with at least one dose of HPV vaccination. The number of women completed three doses of vaccination were 296,595, representing 77.3% of vaccinated women. Looking at the age at vaccination initiation, 293,977 (76.6%) of the vaccinated women initiated vaccination before age 17, and 89,717 (23.4%) initiated vaccination at age 17 and above.

During the follow-up period, we observed 19 cases of invasive cervical cancer among vaccinated women (mean follow-up: 5y) and 538 cervical cancer cases among unvaccinated women (mean follow-up: 6.6y). The cumulative incidence proportion before age 20 was extremely low, but showed a sudden increase of cervical cancer for both vaccinated and unvaccinated women at age 23 (Figure 6.6). Onwards, the increase of cumulative incidence proportion among unvaccinated women was greater compared to vaccinated women, and it was constantly higher in unvaccinated than vaccinated women by age 31.

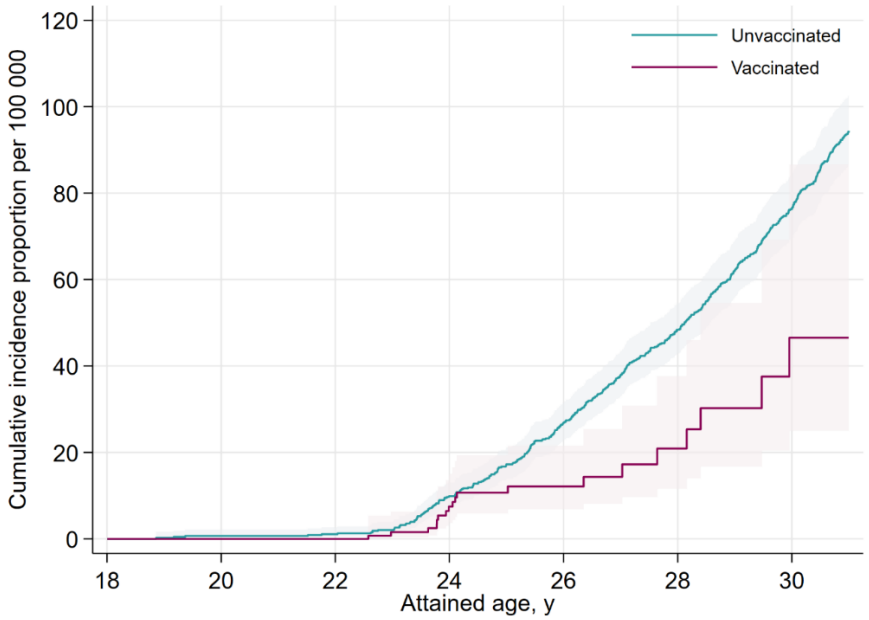

Figure 6.6 *Cumulative incidence proportion of invasive cervical cancer by HPV vaccination status*. HPV, human papillomavirus. Fig. 2 in paper IV.

The crude IR of invasive cervical cancer for unvaccinated women was 6.65 (95% CI, 6.12 - 7.24) while it was 1.00 (95% CI, 0.64 - 1.56) for vaccinated women. This led to an age-adjusted IRR of 0.50 (95% CI, 0.32 - 0.80) for vaccinated women compared to unvaccinated women (Table 6.2). Using Poisson regression model, we also adjusted for other covariates including county of residence and parental characteristics. The results showed the incidence among

vaccinated women was 52% (IRR 0.48, 95% CI 0.30 - 0.76) lower compared to unvaccinated women. Stratifying by age at vaccination initiation, we observed a more pronounced risk reduction among women initiating vaccination before age 17 with a reduction of 83% (IRR 0.17, 95% CI 0.04 - 0.69), while for women initiating at age 17-30 we observed a risk reduction of 40% (IRR 0.60, 95% CI 0.37 - 0.97).

Table 6.2 *Age at HPV vaccination initiation and incidence rate ratios (IRRs) of invasive cervical cancer.* Table 2 in paper IV.

HPV vaccination	No. of cases	Person-time (years)	Crude incidence rate (95% CI)[a]	Age-adjusted IRR (95% CI)	Adjusted-IRR (95% CI)[b]
Unvaccinated	538	8,084,415	6.65 (6.12 - 7.24)	Ref.	Ref.
Vaccinated	19	1,906,704	1.00 (0.64 - 1.56)	0.50 (0.32 - 0.80)	0.48 (0.30 - 0.76)
Vaccinated					
age<17	2	1,338,440	0.15 (0.04 - 0.60)	0.18 (0.04 - 0.73)	0.17 (0.04 - 0.69)
age 17-30	17	568,263	2.99 (1.86 - 4.81)	0.63 (0.39 - 1.02)	0.60 (0.37 - 0.97)
Vaccinated					
age<20	12	1,768,836	0.68 (0.39 - 1.19)	0.51 (0.28 - 0.91)	0.48 (0.26 - 0.86)
age 20-30	7	137,868	5.08 (2.42 - 10.65)	0.50 (0.24 - 1.05)	0.48 (0.22 - 1.01)

a. Per 100,000 person-years

b. Adjusted for age as a spline term with 3 degree of freedom, county of residence, mother's country of birth, highest parental educational level, highest annual family income level, mother's cervical intraepithelial neoplasia grade 3 or worse (CIN3+) history, and mother's previous diagnosis of cancers other than cervical cancer.

7 DISCUSSION

7.1 METHODOLOGICAL CONSIDERATIONS

7.1.1 Study design

The case-control study design is considered to be time and resource saving, but compromises with loss of efficiency compared to a full-cohort design. In paper I, we used the nested case-control study design, and selected controls using incidence density sampling within a dynamic cohort, meaning the controls were selected in proportion to their contribution to the person-time at risk (138). Therefore, the ORs in our study can be interpreted as IRRs (135). Additionally, we have previous evaluated the screening and risk of the two major types of cervical cancer (32, 55) in a nested case-control study. Using the same study design allows us to compare the effect size of screening and risk of different histological types of cervical cancer to some extent, which is of great interest when the knowledge on screening and risk of ASC and RICC is limited. In papers II, III & IV, a population-based cohort design was used as we were interested both in absolute and relative risk between the comparison groups. Cohort studies are usually very costly, time-consuming on data collection and informative censoring can introduce bias. However, given the unique opportunities of rich healthcare registers with routinely collected high-quality data, and the individual linkage possibilities with PIN in Sweden, a population-based cohort design based on virtually complete data excludes recall bias and largely minimize selection bias, misclassification and loss to follow-up.

7.1.2 Confounding

Confounding refers to a situation where the effect of the exposure on the outcome is a mixed with effects of other variables. Confounders are defined as factors related to both exposure and disease, but not an intermediate step in the causal pathway between them (138). Confounding can be controlled at the stage of study design (randomization, restriction, and matching) or during data analysis (regression adjustment and stratification).

7.1.2.1 Age and birth cohort

Age is a major confounder for all research questions in this thesis. The incidence of HPV-related diseases and all-cause mortality are strongly related to age. Birth cohort is strongly associated with HPV vaccination uptake, and somewhat related to incidence of HPV-related diseases. Under a specified observational period, age and birth cohort are exchangeable.

We matched on year of birth when selecting controls in paper I to reduce confounding. Since we used incidence density sampling, matching on year of birth was equivalent to matching by age. All matching factors, including year of birth, were implicitly adjusted for in the conditional logistic regression model. In paper II, age at cancer diagnosis could be both a confounder and effect modifier for association between hrHPV status and prognosis of cervical cancer. We included age as a spline term in the regression model, and additionally stratified the analysis by age groups, with adjustment for age within each stratum, to examine whether the association

varied. When investigating the PPV of cytology in paper III, we adjusted for birth cohort, since we only examined cytology screening tests at age 23. In paper IV, we used attained age as the underlying time-scale, and adjusted for it via a spline term in the analysis. Birth cohort was considered as an interaction term with HPV vaccination status, and we also computed the IRRs for vaccinated compared to unvaccinated women in separate birth cohort strata.

7.1.2.2 Behavioral factors

A major challenge in register-based studies is the absence of information on covariates such as lifestyle factors, smoking, number of sexual partners, and STIs, as these behavioral factors are not easily available from health-care registers. We attempted to minimize the confounding effects through adjustment for proxy factors available from nationwide registers. Controlling for such factors is essential, because they are usually related to health seeking behavior, which corresponds to screening attendance and vaccine uptake in this thesis. Meanwhile, these factors could also influence the risk of cervical diseases and death. Therefore, we retrieved information on educational level (paper I, II & IV) and income level (paper IV) to serve as proxies of lifestyle factors, smoking and STIs, and included them in the regression models to minimize confounding. However, we were to acknowledge that any observational study, even if it is in possession of a rich set of covariates, may be afflicted by residual confounding.

7.1.3 Selection bias

Selection bias is a systematic error derived from selecting subjects from factors that affect study participation. In all papers, we used the entire Swedish population, and therefore, the selection bias that comes from an unrepresentative study population should be limited. However, the selection bias can exist in other forms.

7.1.3.1 Healthy volunteer bias

We examined the impact of preventive strategies including cervical screening and HPV vaccination on the risk of cervical cancer. Individuals attending screening and vaccinating could be generally more health-conscious, leading to so called “healthy volunteer bias”. If so, we would more likely to overestimate the protective effect of screening or HPV vaccination, which applies to paper I and paper IV. We therefore controlled for education in the regression model as a proxy for the health consciousness in order to minimize such a bias in paper I. Furthermore, we assessed the potential healthy volunteer bias by conducting an identical nested case-control analysis examining screening and risk of stomach cancer and rectal cancer as two separate outcomes, which do not benefit from cervical screening. The results showed women participating in cervical screening in the last screening interval had an IRR of 0.91 (95% CI: 0.84 - 0.99) for stomach cancer, compared to women not participating, while the corresponding IRR for rectal cancer was 0.87 (95% CI: 0.82 - 0.91). We concluded that a healthy volunteer effect in terms of screening could account for a general risk reduction of cancer of around 10%, but likely not more than 15%. In paper IV, study participants were mainly vaccinated through

opportunistic or free-of-charge catch-up vaccination program, and healthy volunteer bias could play a role here. In the regression model, we controlled for residential factors and parental characteristics in terms of education, income, country of birth, and previous disease history, and the estimates remained essentially unchanged.

7.1.3.2 Detection bias

Vaccinated individuals were more likely to attend screening (139, 140), which is linked to higher chances for detecting asymptomatic cervical disease as compared to women who did not vaccinated. This could lead to an underestimation of the effectiveness of HPV vaccination in paper IV, because the vaccinated women were more likely to detect otherwise asymptomatic cervical cancer in screening. However, attending screening can also prevent cervical cancer through detection and treatment of precancerous lesions. If vaccinated women had higher screening attendance than unvaccinated women, more cancers might have been prevented through screening in the vaccinated group, which would result in an overestimation of the effectiveness of HPV vaccination. In this case, as cancers occurring before age 31 typically follow more aggressive clinical course, the likelihood of prevention through screening should be limited.

7.1.3.3 Lead time and length bias

Lead time bias and length bias should be noted when evaluating the benefits of screening on survival. Lead time bias means that the screened population could show a falsely improved cancer survival because the cancer is diagnosed earlier through screening before showing symptoms. Length bias refers to the fact that aggressive tumors having a shorter preclinical period than non-aggressive tumors, and screening would be likely to detect non-aggressive tumors than aggressive tumors, leading to a biased estimation of cancer survival.

In paper II, we did not study the benefits of screening on cancer survival directly, but women with hrHPV-positive tumors were more likely to be screen-detected compared to women with hrHPV-negative tumors. If there were differences in the clinical course with regard to tumor HPV status, we suspected hrHPV-positive tumors to be less aggressive and with a longer preclinical time-window to be detected in screening. To minimize this bias, we controlled for cancer stage in the main analysis, which to some extent accounts for these aspects. In addition, we stratified the comparison by mode of cancer detection, and found the difference of prognosis with regard to tumor hrHPV status remained.

7.1.4 Misclassification

7.1.4.1 HPV vaccination

The major source of misclassification in this thesis is misclassification of HPV vaccination status, due to the anonymous registration of vaccination records in SVEVAC. In the opportunistic vaccination period (2006-2011), individuals could receive the reimbursement

only if the vaccines were prescribed, so that the vaccination would be registered in the PDR. We used this information to complement vaccination records in both papers II & IV, which should minimize the misclassification of HPV vaccination during that period. However, a small proportion of school-based vaccinations and catch-up vaccinations could not be complemented with information from the PDR in this manner. This mainly applied to vaccinations through school-based programs in 2012, and catch-up vaccinations from 2012 onwards. Misclassifying vaccinated women as unvaccinated will result in an underestimation of PPV changes for vaccinated compared to unvaccinated women (paper III) and underestimation of the effectiveness of HPV vaccination (paper IV).

7.1.4.2 Invasive cervical cancer

We carefully reviewed medical charts with additional histopathological reviews to ensure the diagnosis of all cervical cancer in the National Cervical Cancer Audit Database. Cases which were not primary, invasive, epithelial (e.g. sarcoma, endometrial cancer), or reoccurrent cases were excluded. Therefore, misclassification of invasive cervical cancer in this scenario was highly unlikely. However, to include those cases would introduce misclassification. This could potentially dilute the association between screening and risk of ASC and RICC, and bias the distribution of HPV types in paper I. Meanwhile, this would also increase the case load of hrHPV-negative tumors, meaning our estimates could be biased in either directions in paper II.

7.1.4.3 Tumor HPV status

Besides from mistakenly classifying non-cervical cancer as cervical cancer, another argument against HPV-negative cervical cancers was the laboratory tests were simply not sensitive enough to detect HPV types in some tumors, which would lead to misclassification. This could be due to poor quality of samples or inefficient amplification of the test. However, using an identical laboratory method in paper II, we detected HPV in 97% of cases diagnosed as CIN3+ in a laboratory Audit (141). So we consider misclassification due to technical issues being highly unlikely, but if true, the difference on prognosis with regard to tumor hrHPV status will be attenuated and towards the null.

7.1.4.4 Incident and prevalent cervical cancer

Due to absence of information on HPV infection at the time of HPV vaccination in paper IV, we were not able to distinguish incident cases (HPV- naïve at the time of vaccination) and prevalent (prior HPV infection by time of vaccination) cervical cancer cases. As the HPV vaccines in use does not have therapeutic effect for the existing HPV infection (142, 143), inabilities to distinguish prevalent and incident cervical cancer cases in the vaccinated group will result in an underestimation of the effectiveness of HPV vaccination, especially for prior infection of HPV16/18. Therefore, we introduced a buffer period by adding a lag time after the first dose of vaccination to account for the prevalent cases, and minimize the bias of our estimation (Supplementary table S3 in paper IV).

7.1.5 Generalizability

Generalizability, also called external validity, measures to which extent findings could be applicable to other settings. Among all findings in this thesis, results on screening and reduced risk of ASC and RICC should be able to generalize to most countries with screening programs. However, because the sensitivity of the cytology test can vary by country (144), our results on magnitude of risk reduction might only be applicable in certain countries with organized screening programs, comparable quality of cytology and clinical management. Similarly, the absolute value of PPV of cytology for CIN2+ should be generalized with caution, accounting for the sensitivity of cytology. However, we provided a measure of PPV change on a relative scale by age at vaccination initiation, which to some extent increased the generalizability. Besides, effectiveness of the HPV vaccination against cervical cancer can be relevant to all countries with HPV vaccination programs. Considering we have only followed women up to age 31, the current effectiveness can only be generalized to younger women. Last but not least, prognostic utility of tumor hrHPV status for prognosis of cervical cancer should be possible to generalize in a wider range of settings, regardless of whether the association is actually causal, or whether hrHPV status is a proxy for incompletely known but relevant tumor biology.

7.2 INTERPRETATION AND IMPLICATIONS

7.2.1 Cervical screening and risk of adenosquamous cell carcinoma and rare invasive cervical carcinoma.

We found that routinely attending cervical screening could significantly reduce the risk of both ASC and RICC, and the risk reduction was more pronounced for women who had two screening tests during the last two recommended screening intervals than women who had only one test in one of the two recommended intervals. This finding highlighted the importance of attending cervical screening regularly and adhering to the recommended intervals to acquire benefits of preventing ASC and RICC. Meanwhile, the attenuated risk reduction for RICC compared to ASC in women who had similar screening attendance might reflect 1) distinctive rapid progression of RICC and 2) RICC were more likely to be preceded by glandular lesions which increased the difficulties of sampling because they located in the endocervical canal. Subsequently, the probabilities of detecting abnormal cells in screening with cytology reduced.

Considering the aggressiveness of those types of cervical cancer, one could argue that highly aggressive cancers (especially RICC) are not expected to have precursors, and therefore might not fulfil screening criteria (145). We, however, showed that there was a risk reduction for both ASC and RICC through cervical screening. The risk reduction for ASC and RICC attributed to cervical screening is comparable to squamous cell carcinoma and adenocarcinoma (Table 7.1). The risk reduction of ASC is similar to squamous cell carcinoma, and the magnitude of risk reduction is slightly less for RICC and adenocarcinoma. This indicates that there is likely a precursor stage for most ASC and RICC, and therefore, can be prevented by cervical screening.

Table 7.1 *Incidence rate ratios (IRR) of cervical carcinoma by screening status and histological types.* Supplementary table F in paper I.

Screening status	SCC (n=2902) IRR[a] (95% CI)	AC (n=766) IRR[a] (95% CI)	ASC (n=155) IRR[a] (95% CI)	RICC (n=152) IRR[a] (95% CI)
No test	Ref	Ref	Ref	Ref
One Test	0.43 (0.39 - 0.47)	0.88 (0.71 - 1.08)	0.39 (0.26 - 0.59)	0.69 (0.45 - 1.06)
Two tests	0.19 (0.17 - 0.21)	0.57 (0.46 - 0.71)	0.22 (0.14 - 0.34)	0.34 (0.21 - 0.55)

SCC: squamous cell carcinoma; AC: adenocarcinoma; ASC: adenosquamous cell carcinoma; RICC: rare types of invasive cervical carcinoma. Only women age 30 and above were included. [a] Incidence rate ratio adjusted by education level and age. Data for SCC and AC adapted from Wang et al, IJC 2019 (55).

A stronger magnitude of risk reduction from having two tests was observed and the results confirmed the commonly accepted fact that repeat testing could potentially increase the sensitivity of cytology. For women who had two normal tests in the last two recommended screening intervals, a very low risk of ASC and RICC was demonstrated. In contrast, the higher risk of ASC and RICC after having at least one abnormal test could be due to inadequate clinical management, in addition to lower sensitivity of cytology for glandular cells. A previous publication based on the nationwide Audit in Sweden showed that women who had abnormal cytology but without a follow-up biopsy was associated with 89% significantly increased risk of cervical cancer compared to those who had follow-up biopsies (32). Moreover, it might also reflect that the current management strategies might need improvement for certain abnormal cytology (ie. atypia glandular cells) (146, 147).

Considering that the majority of ASC and RICC were positive for hrHPV, with HPV18 as the dominant type, we could expect that most of the ASC and RICC can be prevented through HPV vaccination. HPV testing has been shown to detect early precancerous lesions, but how the HPV testing will influence ASC and RICC is still to be investigated. The established nationwide cervical cancer Audit framework in paper I using a nested case-control study design could serve as a standard for evaluation of cervical cancer screening. This should also be applicable to countries without information on cervical screening readily available.

7.2.2 Tumor high-risk HPV status and prognosis of invasive cervical cancer.

Women who had hrHPV-positive tumors were associated with better prognosis of cervical cancer compared to women who had hrHPV-negative tumors. Of note, hrHPV-negativity in the tumor does not necessarily mean that hrHPV has not been involved during the development of cervical cancer. On the contrary, hrHPV-negativity in representative tumor tissue might indicates the loss of hrHPV-expression during the carcinogenic process in a subset of women. A previous study showed that the proportion of HPV-negative tumors increased among women diagnosed at an older age and more advanced stage (148), which was also confirmed in our data. However, the tumor biology of how the loss or integration of hrHPV may occur, and the mechanisms for why women who had hrHPV-positive tumors showed better prognosis compared to women who had hrHPV-negative tumors is not completely clear.

The hrHPV-negative cancers may have been immortalized and lost internal mutation control. This might have progressed to the point of acquiring significant somatic host mutational burden, which is connected to malignant potential for growth and spread. More work is needed to further elucidate potential detailed explanations and mechanisms. According to the current evidence on HPV and the prognosis of oropharyngeal cancer, HPV-positive cancers could be more immunogenic due to the presence of viral proteins, so the tumors could become more susceptible to the immune system and be better controlled (149). One could speculate that this is also the case for hrHPV-positive cervical cancer.

Although our data in this study was not able to provide constructive explanations to this biological phenomenon, we suggested a significant association between detection of hrHPV in tumor tissue and prognosis of cervical cancer. The difference in prognosis between hrHPV-positive cases and hrHPV-negative cases was robust and independent of age at cancer diagnosis, FIGO stage, and histological types. Current laboratory methods in HPV genotyping and testing for tumor hrHPV status could possibly provide extra insights beyond the currently established clinical prognostic factors.

7.2.3 Screening performance of cytology test among HPV-vaccinated cohorts.

The lower PPV for CIN2+ in vaccinated compared to unvaccinated women among birth cohorts that were partially vaccinated through either subsidized opportunistic or free-of-charge catch-up vaccination confirmed earlier findings from simulation studies that PPV was predicted to decrease when the prevalence of cervical lesions declined (91, 94). Therefore, it is not surprising to see a lower PPV among girls who initiated vaccination before 17, which is well in line with what the protection of the HPV vaccine confers. Our findings highlighted that the PPV for the screening program did not only depend on vaccine coverage in the respective birth cohort, but also relied on the proportion of individuals vaccinated at young age - an important factor to be accounted for when considering changing of screening strategies in real-life.

The vaccination coverage has increased substantially among younger birth cohorts in Sweden. Over 80% of females in birth cohorts 1999 onwards were vaccinated (70). With a coverage over 50% in girls, the birth cohorts should also benefit from a herd-immunity effect (90), leading to a very low prevalence of cervical lesions in that population. Consequently, a very low PPV of cytology for CIN2+ could be expected, when the younger birth cohorts enter the screening program.

Given the proven effectiveness of the HPV vaccines, HPV16/18-related lesions in highly vaccinated birth cohorts would also be expected to be less common, and possibly with an increased load of low-grade cytology caused by non-oncogenic HPV types. As we saw, the PPV of low-grade cytology for CIN2+ also declined in the vaccinated population. A pragmatic adaptation of the screening strategies will be needed and should account for shifts vaccination coverage by cohort. HPV16/18 positivity at first screening among highly vaccinated cohorts

could serve as an indication of persistent infection with HPV16/18 (an infection acquired before HPV vaccination), therefore HPV-based screening with genotyping might be an alternative in achieving better performance.

7.2.4 HPV vaccination and risk of invasive cervical cancer.

The ultimate goal of HPV vaccination is to prevent invasive cervical cancer. We are, to date, the first to show HPV vaccination could effectively reduce the risk of invasive cervical cancer among vaccinated women compared to unvaccinated women, based on a nationwide real-life vaccination program. The results were in line with available data including a promising clinical trial (150) which reported no detection of HPV-related cancer cases after HPV vaccination, and an ecological study from US (151) that showing a decreased cervical cancer incidence among young women after implementation of HPV vaccination.

In the assessment of comparing age at vaccination initiation with regard to incidence of cervical cancer, a more pronounced protection against cervical cancer was seen among women initiating vaccination before age 17. This finding emphasized the benefits of administrating HPV vaccination at younger age in order to achieve good protection and greater effectiveness. The incubation time from HPV infection to occurrence of cervical cancer usually takes 5 to 20 years (25). As we have only been able to assess the incidence of cervical cancer among women up to age 30, the effectiveness of HPV vaccination derived from our data might mainly imply the effectiveness of HPV vaccination against fast-growing tumors among young women.

In our population, only part of the birth cohorts included (1993 onwards) achieved a coverage above 50%, the threshold for achieving a herd-immunity effect (90). However, with an interaction analysis, we did not detect any herd effect in the current population. This could possibly be due to the relatively short follow-up for the younger birth cohorts as they have not yet been followed up to the age at which the incidence of cervical cancer increases. With that being said, considering herd effects is essential in future evaluations of long-term effectiveness of HPV vaccination, especially in birth cohorts vaccinated at young ages and with high vaccine coverage. Otherwise, the effectiveness of HPV vaccination would be underestimated.

8 CONCLUSION

With the included papers in this thesis, a few conclusions could be noted:

- Cervical screening was associated with a substantially decreased risk of both ASC and RICC, and the risk reduction derived from screening was attenuated for RICC compared to ASC. The majority of ASC and RICC tumor tissues were positive for high-risk HPV. The evidence provides a benchmark for evaluation of future cervical screening strategies (paper I).

- hrHPV status in cervical tumor tissues was associated with prognosis of invasive cervical cancer. Women with hrHPV-positive cervical tumors showed a significantly better prognosis than women with hrHPV-negative tumors, and the difference was independent of age, FIGO stage, and histological type. Rountinely testing HPV genotypes in cervical tumors could add value to the clinically established prognositic factors (paper II).

- PPV of cytology for CIN2+ decreased among women who had HPV vaccination, and the the maximum reduction was seen among women vaccinated before age 17 followed by women vaccinated at age 17-22. A continued decrease of PPV in cytology-based screening could be expected, especially among highly vaccinated birth cohorts, which points towards the need to re-evaluate screening methods and requirements for the young and vaccinated population (paper III).

- Effectiveness of HPV vaccination against invasive cervical cancer in a nationwide population vaccinated through a real-life program was demonstrated. HPV vaccination is associated with lower risk of invasive cervical cancer at population level. (paper IV).

9 FUTURE PERSPECTIVES

Globally, there were approximately 570,000 newly diagnosed cervical cancer cases and 311,000 deaths that occurred in 2018 (152). In May 2018, WHO called for the elimination of cervical cancer as a public health problem (age-adjusted incidence rate below four per 100,000 women-years) within this century (19). Australia has been predicted to eliminate cervical cancer by 2028, if the current primary HPV screening and HPV vaccination maintained (153). Recently, WHO has proposed a draft of global strategy with "90-70-90 target" (90% girls fully vaccinated before age 15, 70% women screened at age 35-45, and 90% women treated for cervical diseases) by 2030 for countries on their path towards elimination of cervical cancer (154). Modelling studies showed that achieving the target is expected to significantly prevent deaths and reach the goal of elimination of cervical cancer in low- and middle- income countries within the next century (155, 156). Therefore, a chief goal in the future should be to minimize the disparities between high-income and low- and middle- income countries, and to scale-up the coverage of preventive strategies globally.

This thesis addresses a range of research questions on the role of HPV, HPV vaccination, and cervical screening. HPV primary screening has been gradually implemented in many countries. Our findings on cervical screening with cytology and the reduced risk of ASC and RICC set a benchmark for future evaluation on organized screening program, especially after the transition from cytology screening to primary HPV screening. A regular monitoring and evaluation would allow us to ensure the quality of organized screening program, and to identify the issues for further improvement. Despite that a negative HPV test provides greater reassurance against CIN2+, primary HPV screening has its own challenges. Women who had HPV-positive test results usually receive a triage test to ensure those having transient HPV infection will not be referred to colposcopy. The current triage methods are mainly partially HPV genotyping and cytology, and other options such as p16/Ki-67 dual stain cytology and methylation are also under consideration (157). What will be the optimal triage method under the current screening strategy to allow quick stratification of women at different risk levels needs further investigation. An effective triage method could help to avoid unnecessary referral and treatment, which might result in harms (e.g. adverse pregnancy outcomes) from screening.

In Sweden, HPV vaccination was introduced more than 10 years ago and with demonstrated population impact. In the short-term, HPV genotyping of HPV-vaccinated cancer cases to understand reasons for the occurrence of those cancers is needed. A follow-up study to investigate the long-term effectiveness of HPV vaccination against cervical cancer would be essential, and it could also provide more insights on the duration of protection from HPV vaccines. Effectiveness of HPV vaccination with regard to number of doses will also be of great value in enhancing the cost-effectiveness of the vaccination program. Together with the gender-neutral vaccination program, which has been implemented in certain countries and soon will be in Sweden, evaluation on herd effects and monitoring the HPV genotypes circulating in the populations could also be strategically important. Besides, a regular surveillance of

adverse events after HPV vaccination is important to ensure the long-term safety profile of HPV vaccination, and also strengthen the public confidence in the vaccination program.

Furthermore, with improving vaccination coverage and changing HPV prevalence in the population due to highly effective vaccines, reconsidering screening methods for vaccinated cohorts, and the target age of starting screening are crucial questions to consider. In Australia, HPV screening has been used as primary screening for birth cohorts where the majority received vaccination, and it seems that HPV primary screening would be more optimal than cytology for highly vaccinated cohorts. A pilot study of using primary HPV screening among women under 30 will be implemented in Sweden later this year. With the development of personalized medicine, will risk stratified personalized screening be applicable? There are lots of work ongoing and upcoming results to look forward to.

Although the benefits from HPV vaccination and screening are demonstrated, and a high coverage of HPV vaccination (over 80% for at least one dose in the school-based program) and screening (over 80% coverage for one screening interval) is documented in Sweden, we should note that there is potentially a group of women who have not participated in either of these preventive procedures. According to the current guidelines, vaccinated women are recommended to attend screening. Previous studies also demonstrated that vaccinated women had equivalent or higher screening attendance than unvaccinated women from the opportunistic and catch-up program (139, 140). In the coming years, an updated investigation on screening attendance by vaccination status is warranted to gain further insights on how to promote the coverage of cervical cancer prevention programs.

Last, yet importantly, there has been a heated debate on HPV-negative cervical cancer in recent years. We have shown that tumor hrHPV status could be a potential biomarker for prognosis of cervical cancer, especially when HPV testing has become routinely available. How to integrate this information clinically to support the decision-making in treatment could be further investigated. Besides, it would be very interesting to verify the HPV genotyping results based on fresh tumor tissues, and also link to their previous HPV screening results given that information is routinely collected through NKCx, and more and more counties are introducing HPV screening. This could provide insights on 1) whether HPV-negative cervical cancer was HPV-positive at some point during the screening, which we believe the answers to be yes 2) when and how the HPV causing cervical cancer is lost during carcinogenesis, and subsequently influences the prognosis.

This thesis contributes evidence in prevention and prognosis of cervical cancer. With HPV vaccination, cervical screening and treatment of precancerous lesions, cervical cancer could be eliminated. Efforts in minimizing the disparities in accessing healthcare resources, improving and maintaining a good coverage of preventive strategies at global level are required. Eventually, cervical cancer would be eliminated as a public health problem.

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