Prevention, incidence, and survival of cervical cancer in Sweden

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LUND
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DOCTORAL DISSERTATION

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Abstract

Cervical cancer is preventable by screening. In 1967, Sweden introduced a cervical screening program. Screening for high-risk human papillomavirus (hr-HPV), the causative factor of cervical cancer, is more sensitive than cytology and can be performed as a self-sample. Non-attendance to screening is a large risk factor for cervical cancer. Self-samples may improve screening attendance.

The aims of this thesis were: To analyze if the cervical screening program in Sweden can be improved by using vaginal self-samples and an HPV mRNA assay, with the main focus on screening non-attendees. To obtain knowledge if cervical cancer incidence and survival has changed since the implementation of the screening program.

Study I: Incidence and net survival according to morphology, age, and stage at diagnosis among women diagnosed with invasive cervical cancer between 1960 and 2014 were calculated. The age-standardized incidence of squamous cell carcinoma (SCC) decreased until the year 2000, while the incidence of adenocarcinoma (ADC) increased continuously. Age-standardized 5-year net survival increased. SCC and ADC did not statistically differ in net survival after 2012. Among women ≥75 years, long-term net survival has decreased since 1960.

Study II: The sensitivity and specificity of vaginal and urine self-samples compared to cervical samples analyzed by Aptima HPV mRNA assay were evaluated in a referral population. The sensitivity for detection of high-grade squamous intraepithelial lesions /adenocarcinoma in situ/cancer was 85.5% for the vaginal self-sample, 44.8% for the urinary sample, 100.0% for the cervical sample and 81.7% for cytology.

Study III-V: Screening non-attendees or women in the upper age screening limit were sent a vaginal self-sampling kit by mail. In study III, 1,000 women, aged 69-70 years, received a kit. In study IV and V, 6,023 and 19,766 women, aged 30-70 years, received a kit. Returned samples were analyzed for HPV mRNA by Aptima assay. HPV-positive women were invited to follow-up. The response rate of the self-sample was 43.3%, 13.2% and 18.5% for study III, IV and V respectively. The HPV prevalence was 6.2% in study III, and no cases of high-grade dysplasia/cancer were diagnosed. The HPV prevalence was 9.9% and 11.3% in study IV and V respectively. In study IV, the prevalence of cervical cancer was almost seven times higher compared to organized screening, but in study V the prevalence of cancer was not increased.

Conclusion: This thesis demonstrated that the incidence of SCC, but not ADC, has decreased since 1960. SCC and ADC did not statistically differ in net survival after 2012. The decreased long-term net survival among women ≥75 years of age suggests the need for prolonged HPV screening up to 75 years of age. Self-sampling is a promising method since it was accepted among women 69-70 years old, and it increased the attendance to cervical screening by almost one fifth among non-attendees. A vaginal HPV self-sample analyzed by Aptima mRNA assay showed a similar sensitivity as routine cytology and may be used to reach screening non-attendees. Among screening non-attendees and women in the upper age screening limit, around one in 10-20 tested positive for HPV mRNA, with risk of development of cervical dysplasia, although the prevalence of cervical cancer varied between the studies.

Key words Cervical cancer, Human papillomavirus, Screening, Self-sampling, Incidence, Net survival

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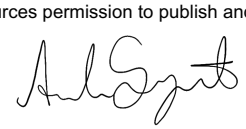
Signature  Date 2021-10-10

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Scientia potentia est

(Knowledge is power)

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List of original papers

This thesis is based on five papers, referred to by Roman numerals below.

I. Time trends for incidence and net survival of cervical cancer in Sweden 1960-2014 – A nationwide population-based study. Sundqvist A, Moberg L, Dickman PW, Högberg T, Borgfeldt C. *Submitted*.

II. Self-sampling with HPV mRNA analyses from vagina and urine compared with cervical samples. Asciutto KC, Ernstson A*, Forslund O, Borgfeldt C. *Journal of Clinical Virology*. 2018 Apr;101:69-73. doi:10.1016/j.jcv.2018.02.002.

III. Detection of HPV mRNA in self-collected vaginal samples among women at 69-70 years of age. Ernstson A*, Asciutto KC, Sturesson J, Norén J, Forslund O, Borgfeldt C. *Anticancer Research*. 2019 Jan;39(1):381-386. doi:10.21873/anticanres.13123.

IV. Cervical cancer prevention among long-term screening non-attendees by vaginal self-collected samples for hr-HPV mRNA detection. Ernstson A*, Urdell A, Forslund O, Borgfeldt C. *Infectious Agents and Cancer*. 2020 Feb 13;15:10. doi:10.1186/s13027-020-00280-0.

V. Promotion of cervical screening among long-term non-attendees by HPV self-sampling. Ernstson A*, Forslund, O, Borgfeldt C. *Journal of Cancer Prevention*. 2021 Mar 30;26(1):25-31. doi:10.15430/JCP.2021.26.1.25.

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The following publication is not included in the thesis but is of relevance to the field:

Equal prevalence of severe cervical dysplasia by HPV self-sampling and by midwife-collected samples for primary HPV screening: a randomized controlled trial. Hellsten C, Ernstson A*, Bodelsson G, Forslund O, Borgfeldt C. *European Journal of Cancer Prevention.* 2021 Jul 1;30(4):334-340. doi:10.1097/CEJ.0000000000000693.

* The former last name of Avalon Sundqvist was Ernstson.

Abstract

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Summary in Swedish - Sammanfattning på svenska

Livmoderhalscancer är den fjärde vanligaste cancerformen bland kvinnor i världen och den andra vanligaste bland kvinnor 15–69 år. Detta till trots att majoriteten av alla livmoderhalscancerfall går att förebygga med hjälp av välfungerande screening.

I stort sett all livmoderhalscancer orsakas av det sexuellt överförbara viruset humant papillomvirus (HPV). Cirka 75% av världens befolkning infekteras någon gång i livet med HPV och de flesta får inga besvär. Det finns emellertid en grupp med högrisk HPV (hr-HPV) som i sällsynta fall kan ge uppkomst till cancer. Bland de som infekteras med hr-HPV läker 90% ut infektionen inom 6–18 månader. Om infektionen blir kvarstående finns dock en risk att cellförändringar på livmodertappen uppstår vilket på lång sikt kan ge upphov till cancer. Lätta cellförändringar läker i regel ut spontant men svårare cellförändringar behöver tas bort för att förhindra utveckling till cancer. Screening mot livmoderhalscancer syftar till att hitta cellförändringar i tid, behandla dessa vid behov och förhindra att cancer uppstår.

Sverige var ett av de första länder i världen att införa ett nationellt screeningprogram mot livmoderhalscancer vilket upprättades i slutet av 1960-talet. Idag kallas alla kvinnor i åldrarna 23–64 år till screening med cellprov. Tidigare utfördes cytologisk analys (analys av celler) på alla cellprov men sedan 2015 rekommenderas i stället HPV-analys för alla kvinnor över 30 år då detta har visat en bättre känslighet för att hitta cellförändringar. Det finns flera olika HPV-analyser tillgängliga, de två stora grupperna är HPV-analyser baserade på DNA- eller mRNA-teknik. Analyser baserade på mRNA-teknik har visat sig vara något mer specifika än DNA-analyser. En fördel med HPV test är att de går att utföra som självtest som kvinnan kan ta i sitt hem och med vanlig postgång skicka in provet till laboratoriet. Att inte delta i screeningen är idag en av de största riskfaktorerna att drabbas av livmoderhalscancer. Självtest är en potentiell metod för att få fler kvinnor att delta i screeningprogrammet.

Det finns olika typer av livmoderhalscancer, den vanligaste formen är skivepitelcancer följt av adenokarcinom. Sedan införandet av screeningprogrammet har antalet livmoderhalscancerfall i Sverige minskat med cirka 50%. Under de senaste åren har dock en ökning setts. I flera länder har det även noterats att antalet adenokarcinom har ökat.

Syftet med denna avhandling var att analysera om incidensen och överlevnaden av livmoderhalscancer i Sverige har förändrats sedan införandet av ett screeningprogram. Syftet var även att utvärdera om screeningprogrammet för livmoderhalscancer i Sverige kan förbättras genom användning av vaginalt självtest analyserat med en HPV mRNA analys, med särskilt fokus på de kvinnor som i dagsläget ej deltar i screening.

Studie I: I delstudie I analyserades incidensen och netto-överlevnaden av livmoderhalscancer i Sverige mellan åren 1960–2014. I analyserna tittade vi separat på skivepitelcancer och adenokarcinom, olika åldersgrupper samt olika stadium av sjukdomen. Vi fann att incidensen av skivepitelcancer har minskat fram till år 2000, för att sedan stagnera, och år 2014 sågs en ökning. Incidensen av adenokarcinom har kontinuerligt ökat sedan år 1960. Netto-överlevnaden för livmoderhalscancer har förbättrats sedan år 1960 och de senaste åren är överlevnaden jämförbar för skivepitelcancer och adenokarcinom. Bland våra äldsta kvinnor över 75 år kunde vi dock se en försämrad netto-överlevnad under hela tidsperioden. Högre ålder och högre stadium vid diagnos resulterade i en sämre överlevnad.

Studie II: I den andra delstudien studerade vi känsligheten att hitta allvarliga cellförändringar för ett HPV-självtest taget från urin och vagina i jämförelse med ett barnmorske-/läkartaget prov från livmodertappen. Alla HPV-proverna analyserades med en mRNA analys (Aptima). Tvåhundranio kvinnor kallade till uppföljning på Kvinnokliniken i Lund deltog i studien. Resultaten visade att ett prov taget från livmodertappen analyserat med mRNA analys var 100% känsligt för att hitta allvarliga cellförändringar. Ett självtaget prov från vagina hade en känslighet på 85,5% vilket är jämförbart med känsligheten för cytologisk analys. Urinprovet uppvisade en känslighet på 44,8% vilket anses för lågt för att kunna användas i screening.

Studie III: Medelåldern bland kvinnor i Sverige stiger och cirka en femtedel av alla livmoderhalscancerfall diagnostiseras hos kvinnor över 75 år. En ökad kunskap kring HPV-infektion och screeningmöjligheter bland äldre behövs. I delstudie III undersökte vi hur många som returnerade ett vaginalt HPV-självtest bland 1000 kvinnor, 69–70 år, i Lunds kommun, som inte hade lämnat ett cellprov på fem år eller mer. Vi undersökte även förekomsten av hr-HPV-infektion och cellförändringar bland deltagarna. Det var 43,3% som returnerade sitt självtest och 6,2% av dessa hade hr-HPV infektion. Alla kvinnor positiva för HPV kom på uppföljning hos en barnmorska där inga allvarliga cellförändringar hittades.

Studie IV-V: I de sista två delstudierna undersökte vi möjligheten att skicka ut ett HPV-självtest till kvinnor 30–70 år, i Skåne, som inte hade lämnat ett cellprov på sju år eller mer (icke-deltagare i screening). I delstudie fyra skickades 6023 självtest ut och i delstudie fem 19 766. Andelen kvinnor som returnerade sitt självtest var 13,2% i studie fyra och 18,5% i studie fem. Det var 9,9% respektive 11,3% av dessa som hade hr-HPV infektion. Av de HPV-positiva kvinnorna deltog 83,5% respektive 85,7% på uppföljning hos en barnmorska. Andelen allvarliga cellförändringar vid uppföljningen var inte ökad jämfört med kvinnor som regelbundet deltar i screening. I delstudie fyra hittades dock en nästan sju gånger ökad förekomst av livmoderhalscancer, delstudie fem hade emellertid ingen ökning av livmoderhalscancer.

Dessa studier visar att vi överlag har haft en bra effekt av screeningprogrammet på förekomsten av skivepitelcancer, men screeningprogrammet har inte lyckats minska förekomsten av adenokarcinom. Vi kan se att överlevnaden för livmoderhalscancer har ökat men bland kvinnor över 75 år ses en relativ försämring sedan år 1960, vilket indicerar att den övre åldern för screening kan behöva höjas till 75 år. Äldre kvinnor accepterade HPV självtest i en hög utsträckning vilket är positivt. Bland icke-deltagare i screening ökade HPV-självtest deltagandet i screening med nästan en femtedel i vår sista delstudie, vilket är ett steg i rätt riktning. En högre andel av icke-deltagarna var infekterade med hr-HPV i jämförelse med kvinnor som regelbundet går på screening, vilket indikerar en ökad risk för cellförändringar och på sikt livmoderhalscancer samt belyser vikten att nå dessa kvinnor. Ett vaginalt HPV-självtest analyserat med mRNA analys anses vara känsligt nog för att användas till att nå kvinnor som inte går på vanlig screening, ett HPV-urintest analyserat med mRNA analys rekommenderas dock inte i nuläget på grund av för låg känslighet.

List of abbreviations

ACG: Atypical glandular cells

ADC: Adenocarcinoma

AIS: Adenocarcinoma in situ

ASC-H: Atypical squamous cells cannot exclude HSIL

ASCUS: Atypical squamous cells of undetermined significance

CIN: Cervical intraepithelial neoplasia

FDA: U.S. Food and Drug Administration

FIGO: International Federations of Gynecology and Obstetrics

HLA: Human leukocyte antigen

HPV: Human papillomavirus

hr-HPV: High-risk human papillomavirus

HSIL: High-grade squamous intraepithelial lesion

ICCS: International Cancer Survival Standard

LBC: Liquid-based cytology

LEEP: Loop electrosurgical excision procedure

lr-HPV: Low-risk human papillomavirus

LSIL: Low-grade squamous intraepithelial lesion

NKCx: Swedish National Cervical Screening Registry

NPV: Negative predictive value

PPV: Positive predictive value

Pap test: Papanicolaou test

RS: Relative survival

SCC: Squamous cell carcinoma

SCJ: Squamocolumnar junction

SCR: Swedish Cancer Registry

TZ: Transformation zone

WHO: World Health Organization

Introduction

In November 2020 the World Health Organization (WHO) presented a strategy for elimination of cervical cancer in the future. The effects of cervical screening, vaccination against high-risk human papillomavirus (hr-HPV), the causative factor for cervical dysplasia and cancer, and adequate treatment have the potential to reduce cervical cancer cases below four new cases per 100,000 women per year which is considered as elimination of cervical cancer as a public health problem (1). However, at this pace, global elimination of cervical cancer is predicted by the year 2120, and continuous work on preventing cervical cancer is needed in order to reach elimination (2).

Human papillomavirus

In the 1970s and 1980s, the German virologist Harald zur Hausen demonstrated the role of human papillomaviruses (HPV) in the pathogenesis of cervical cancer and was able to isolate HPV 16 and 18, the two most carcinogenic HPV types. For this discovery he was awarded the Nobel Prize in medicine in the year 2008. His findings have had a great impact on the diagnostic tools and primary prevention methods used against cervical cancer, for example they led to the development of the HPV vaccine (3).

Biology

HPV belongs to the family Papillomaviridae (4) and more than 200 different types have been discovered (5). HPV infects stratified epithelium in genital- or oral mucosa and skin and there are five genera (alpha, beta, gamma, mu and nu) that can infect humans. Viruses associated with the development of mucosal lesions belong to the alpha genus, which can be further classified into low-risk HPV (lr-HPV) types causing benign lesions and hr-HPV types associated with the development of several cancers (6, 7). There are 12 types of hr-HPV classified as carcinogenic (HPV 16, 18, 31, 33, 39, 45, 51, 52, 56, 58 and 59), one as probably carcinogenic (HPV 68) and seven as possibly carcinogenic (HPV 26, 53, 66, 67, 70, 73 and 82) (8).

The target tissue for HPV infection are undifferentiated keratinocytes in the basal lamina of the stratified epithelium. At the cervix it is believed that the virus can directly reach the target cells through the transformation zone. The HPV life cycle follows the cell differentiation of the keratinocytes. The viral genome is transferred to the nucleus of infected cells and when basal cells are moving to the suprabasal epithelial layers the virus starts to replicate. During terminal differentiation of the keratinocytes in the upper layers of the epithelium, viral genome amplification occurs. To allow viral genome amplification, the two oncogenes, E6 and E7, play a key role in maintaining cellular conditions that allow cell proliferation. E6 mediates degradation of p53 to overcome cellular apoptotic processes and E7 deactivates the tumor suppression protein retinoblastoma to stimulate cell-cycle re-entry. With the help of L1 and L2 proteins the amplified genomes form virions which are released through natural tissue desquamation and can initiate a new infection. It is also believed that the protein E4 contributes to virion release. In low-risk HPV infection there is a lower expression of the proteins E6 and E7, allowing cell-cycle re-entry but not cell proliferation in the upper layers of the epithelium, while in hr-HPV infections the expression of E6 and E7 is increased and cell proliferation is mediated in the lower and middle layers of the epithelium, causing neoplasia. Furthermore, in neoplastic progression, the HPV DNA is often integrated in the host chromosome (7, 9, 10).

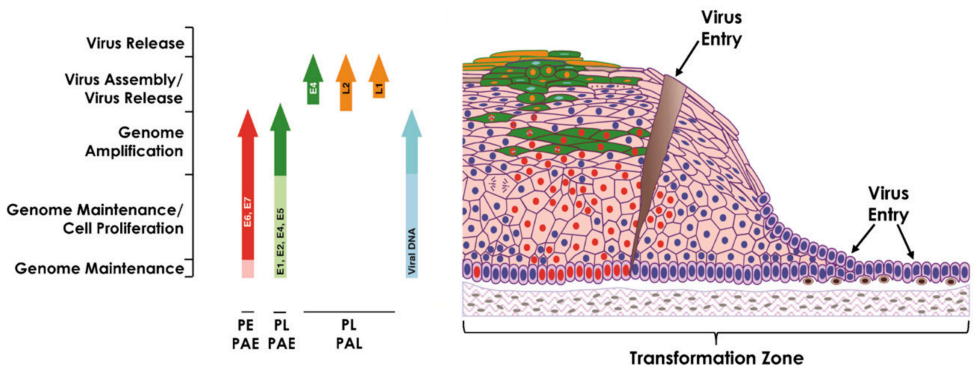

Figure 1. Life cycle of high-risk HPVs in cervical epithelium. HPV: Human papillomavirus. Reprinted from *Doorbar et al. 2012* (9), with permission from Elsevier.

Epidemiology

HPV is the most common sexually transmitted disease, and approximately 75% of the global population will receive at least one HPV infection during their lifetime (11, 12). It is spread through skin-to-skin or skin-to-mucosa contact (13). The estimated prevalence of infection with HPV worldwide is 11.7%, but with large regional differences. A higher HPV prevalence is observed among younger women,

with a peak prevalence of 24% among women <25 years and subsequently lower prevalence with increasing age. However, in Africa, Asia, and South America the decline with increasing age is not as prominent and, in some regions, a bimodal curve has been observed (14).

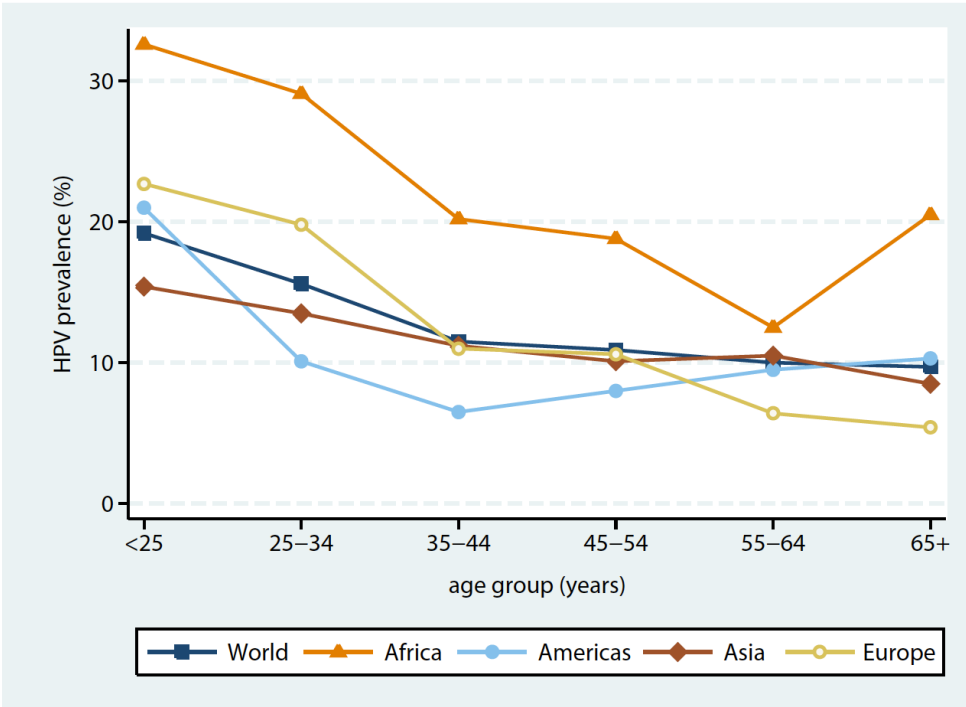

Figure 2. Crude age-specific HPV prevalence (%) in women with normal cervical cytology in the world and its regions. HPV: Human papillomavirus. Reprinted from *Serrano et al. 2018 (14)*, with permission from Elsevier and Oxford University Press.

The most common oncogenic HPV type globally is HPV 16, followed by HPV 18. These are also the most carcinogenic hr-HPV types and are responsible for approximately 70% of all cervical cancer cases worldwide and 50% of cases of high-grade dysplasia (cervical intraepithelial neoplasia 3 (CIN3)). The following most common oncogenic HPV types differ in frequency between continents, although globally they are HPV 31, 33, 35, 45, 52 and 58 (15). In Sweden, HPV 18 and 16 were detected in 57% and 19% respectively of single infections in 2,850 cervical cancer cases diagnosed between 2002 and 2011. The following most common HPV types detected were HPV 45, 31, 33, 52, 39, 70, 56 and 35 (16).

Natural history of HPV infection

The majority of HPV infections do not give any symptoms and around 90% will resolve by cell-mediated immunity in 6-18 months (13). However, a small portion will be persistent infections with HPV, which is a prerequisite for the development of cervical dysplasia and cancer. There is no consensus on the definition of a persistent infection. Today, the most common definition of a persistent HPV infection is detection of the same HPV type at two consecutive controls, but there is no definition of how long the interval should be between the samplings (8). With increased time of persistence, the probability of clearance decreases. One study found that a newly acquired HPV infection had a risk of persistence for a further six months of 37%. For infections that had already been persistent for ≥18 months, the corresponding risk for persistence for six months was 65% (17). With longer persistence the risk of cervical dysplasia or cancer increases (18). The mechanism driving an HPV infection to clearance or persistence is poorly understood. Lifestyle and genetic risk factors for persistent infection have been proposed. Alcohol consumption, smoking, infection with Chlamydia trachomatis and/or the use of oral contraceptives have in some studies shown an increased risk of HPV persistence (19-24), but the results are inconsistent and some studies did not find such a relationship for one or several of the lifestyle factors (22-24). Human leukocyte antigen (HLA) may play a role in HPV persistence and the development toward cancer or regression, depending on the HLA molecules' ability to recognize and bind HPV antigens (7, 25). Furthermore, high viral load, infection with multiple HPV types and/or infection with HPV 16 were found to increase the risk of HPV persistence in some studies, but not all studies found all three of these factors important (23, 24, 26, 27).

Besides the capacity of persistence, there is also increasing evidence that an HPV infection can enter a latent stage with the possibility of reactivation (28). Studies investigating the source of incident HPV infections among middle-age and older women found that a substantial number of incident HPV detections could not be attributed to a new infection. In these studies, 30-85% of incident HPV detections were believed to be due to reactivation of a previous HPV infection, and the likelihood of a newly acquired HPV infection declined with age (29-31). With the knowledge of the possibility of reactivation of a prior HPV infection it is important to study the risk of development of cervical neoplasia or cancer among these women. In a large study by Hammer *et al.* with access to results of repeated HPV testing and co-testing from 1.5 million women it was found that the incidence rates of CIN3+ were 1.5 times higher in women with possible reappearing infection compared to women with a new infection. Furthermore, the study found that cases of CIN3+ attributed to possible reappearing infections or intermittent infections ending with an HPV-positive result were highest among women aged 65 years and older (18). Some studies have found a bimodal distribution of cervical cancer

incidence with a first peak among women around 40 years old and a second peak among older women around 65-79 years old (32, 33). The first peak is generally believed to be related to the start of sexual activity among younger women, with the consequence of new HPV infections. This too could explain the peak among older women since a Swedish study shows increased sexual activity among 70-years-old men and women (34). However, in the study by Hammer *et al.*, only one out of four CIN3+ cases among women ≥65 years could be attributed to a new HPV infection (18). It should therefore be considered that a part of the second peak could be related to reactivated latent infections causing cervical cancer.

HPV-related diseases

Although most HPV infections will not cause any clinical disease, there are important HPV-related diseases. HPV types from the alpha-groups can cause skin-/genital warts, for example HPV 6 and 11 are known to cause 90% of all cases of condyloma (14). Furthermore, in 2012, 4.5% of all new cancers among men and women worldwide were attributed to infection with HPV (14). Cervical cancer is the most common HPV-related cancer, of which 99.7% of all cases of cervical cancer are caused by persistent infection with hr-HPV (35). It is also known that around 88% of anal cancer, 78% of vaginal cancer, 51% of penile cancer and 15-48% (increased percentage with younger age) of vulvar cancer are caused by HPV infection (36). Head and neck cancer is associated with HPV infection, with the strongest association for cancer of the oropharynx (36).

Cervical cancer

Epidemiology

Cervical cancer is the fourth most common cancer among women of all ages worldwide and for women aged 15-69 years it was the second most common cancer after breast cancer in the year 2020. In 2020, the age-standardized incidence of cervical cancer was 13.3 cases per 100,000 women in the world, with large variations across the globe (37). Approximately 84% of cervical cancer cases are diagnosed in low-resource countries. Africa has the highest incidence levels of cervical cancer followed by South America, south-eastern Asia, and central-eastern Europe. Unlike other gynecological cancers, cervical cancer is a disease of younger women, with a top incidence around the age of 40 years in high-resource countries and a global average age at diagnosis of 53 years (38). However, some studies have demonstrated a bimodal distribution with another peak in women aged 65-79 years (32, 33).

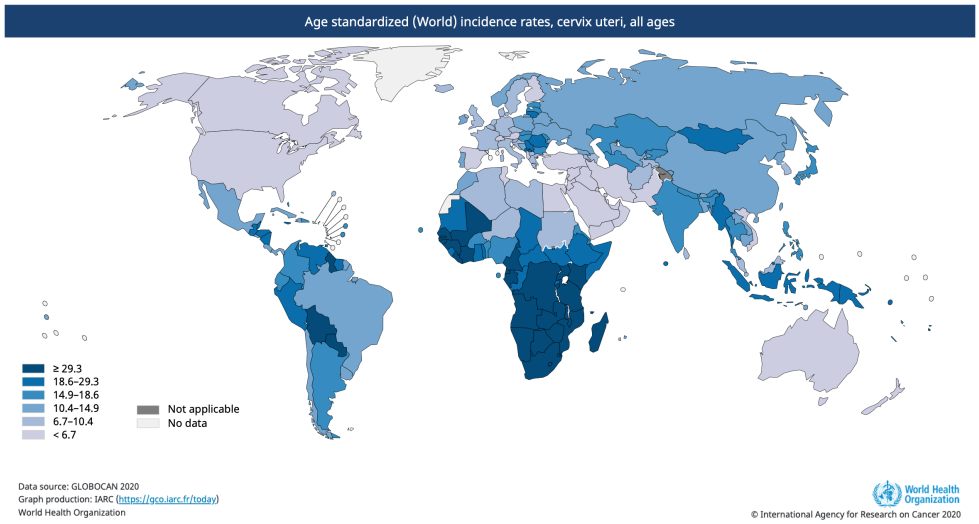

Figure 3. Age-standardized (world standard) incidence rates per 100,000 individuals of cervical cancer across the globe 2020. Source GLOBOCAN 2020 (37).

The majority of cervical cancer cases are considered to be preventable through HPV vaccination and organized cervical screening (39). The Nordic countries were early in implementing screening for cervical cancer, and in Sweden screening was introduced in 1967 and implemented nationwide in 1973 (40). Since then, a large decrease in cervical cancer incidence has been observed, from 15.1 cases per 100,000 individuals in 1970 to 7.9 cases per 100,00 individuals in 2019 (41). However, during the recent decade the decrease in incidence of cervical cancer has stagnated and during 2009-2018 an increase was observed (42, 43). An increased cervical cancer incidence has also been reported in Norway, Finland, and the Netherlands (42, 44-46). In a large re-examination of cytological samples from women diagnosed with cervical cancer in Sweden, it was found that the number of women diagnosed with cervical cancer whose last cervical sample showed normal cytology had increased by 30% (47). Furthermore, studies from Finland have reported an increase in hr-HPV-prevalence during recent decades (48).

Symptoms

Cervical dysplasia and early stages of cervical cancer are often asymptomatic. Clinical symptoms often debut when a visual lesion has developed on the cervix. The most common symptoms, occurring among 70%, are post-coital vaginal bleeding caused by the sensitive surface of the tumor, or metrorrhagia. Changes in vaginal discharge in terms of consistent, thin, malodorous, or blood-streaked discharge occur among 20%. Pain occurs mainly in late stages of cervical cancer

when the tumor has spread to the parametrial tissue and lymph nodes in the small pelvis affecting nerves and surrounding tissue. Spread to the bladder and rectum can also occur in late stages. Late stages can sometimes compress the iliac vessels and cause unilateral leg edema. General symptoms of disseminated cancer such as fatigue, weight loss, decreased appetite and anemia occur (49, 50).

Etiology

The cervix is covered by two cell types, stratified non-keratinizing squamous epithelium on the ectocervix and single layer columnar epithelium on the endocervix. The area for transition between the cell types is called the squamocolumnar junction (SCJ) of the cervix. The location of the SCJ depends on age and hormonal status. Among women of reproductive age and women with increased estrogen levels, due to pregnancy or the use of oral contraceptives, the SCJ is situated on the ectocervix. This will expose columnar cells to the acid environment in the vagina, causing a metaplastic transformation from columnar epithelium into metaplastic squamous epithelium, a new SCJ is created transferring upward toward the endocervical canal. Eventually among older women after menopause the SCJ will be situated in the cervical canal and cannot be visualized on visual inspection. The area between the old SCJ and the new SCJ is referred to as the transformation zone (TZ). The TZ has an important role in understanding the pathogenesis of cervical cancer since the immature metaplastic cells are susceptible to HPV infection (49, 51).

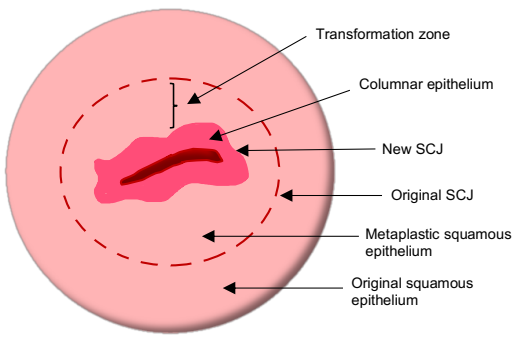

Figure 4. Figure of the cervix showing the squamocolumnar junction (SCJ) of the cervix and the transformation zone. Figure by the author.

Persistent infection by high-risk types of HPV is known to cause 99.7% of all cervical cancer cases (35). On average the progression from incident HPV infection to cervical cancer takes 15-20 years, although among women with immunodeficiency the process can take only 5-10 years (39). The carcinogenesis of cervical cancer includes the development of precancerous lesions. The previous

three-tier nomenclature of precancerous lesions, CIN I-III, has been replaced by the Bethesda system. Squamous intraepithelial lesions are classified as low-grade squamous intraepithelial lesion (LSIL (corresponds to CIN1)) and high-grade squamous intraepithelial lesion (HSIL (corresponds to CIN2-3)). Atypical squamous cells are categorized as atypical squamous cells of undetermined significance (ASCUS), or atypical squamous cells cannot exclude HSIL (ASC-H). Glandular dysplasia is categorized as atypical glandular cells (ACG) and adenocarcinoma in situ (AIS) (52). The carcinogenesis of cervical dysplasia and cancer is not always a straight pathway. HSIL can develop as an early manifestation of HPV infection and cases of LSIL can regress quickly (53). In a study by Castle *et al*. the cumulative three-year incidence of CIN2+ among sexually active women with two consecutive positive HPV results was 17%, if the HPV genotype was HPV 16 the corresponding result was 40.8% (54). In one unethical study from New Zealand which started in the 1960s, the rate of progression from untreated or inadequately treated CIN3 to cervical cancer was studied. This study found an incidence of cervical cancer of 30-50% after 30 years. In comparison, the risk of cervical cancer after 30 years was only 0.7% among women adequately treated for CIN3, which emphasizes the importance of diagnosing and treating high-grade precancerous lesions (55). Although virtually all cervical cancer cases are HPV-positive, there are a small number of cervical cancer cases which do not test positive for HPV. In a nationwide study of 2,850 cervical cancer cases in Sweden, 394 (14%) cases were found to be HPV PCR-negative (16). When adding a deep sequence method 223 cases remained HPV negative (56). Potential reasons for not detecting HPV among cervical cancer cases are: true negative cases which are independent of HPV, HPV genome loss, cancers positive for HPV types not tested for, incorrect cancer diagnosis and failure of HPV detection methods (56).

Co-factors/associated factors

Infection with HPV is common among women worldwide, but most women do not develop cervical cancer. There are several co-factors associated with an increased risk of persistent HPV infection and cervical cancer in the presence of hr-HPV. Early sexual debut and multiple sex partners are known to increase the risk of acquiring an HPV infection and the risk for cervical cancer (57). Increasing number of full-term pregnancies is associated with an increased risk for cervical cancer due to hormonal changes and cervical trauma during vaginal birth causing the TZ to remain on the ectocervix, which increases the susceptibility to infection with HPV (10, 58, 59). Furthermore, the immunosuppression which occurs during pregnancy may favor HPV infection and the hormonal changes could support cervical carcinogenesis (58). Young age at first full-term pregnancy is also associated with increased risk (10, 58).

Use of combined oral contraceptives has been shown to be a risk factor for cervical cancer, with increased risk occurring with increased duration of use. One large re-analysis of several epidemiological studies found a doubled risk of cervical cancer after the use of oral contraceptives for five years or more and no increased risk after ceasing use for ten years or more (60). Combined oral contraceptives are believed to increase the risk for cervical cancer through estrogens and progestogens interacting with hormonal receptors which enhance HPV gene expression in the cervix (10, 61).

Current smoking increases the risk of SCC, but not of ADC. The number of cigarettes smoked per day and younger age at debut of smoking have been found to be associated with increased risk of SCC among current smokers (62). A possible mechanism through which smoking increases the risk of SCC is through immunosuppressive effects which increase the risk of persistent cervical infections and DNA damage in squamous epithelial cells caused by the chemicals in cigarettes (10, 63).

The sexually transmitted diseases Chlamydia trachomatis and Herpes simplex 2 are associated with increased risk of cervical cancer (10) due to the inflammatory response at the cervix leading to genetic damage (64). Furthermore, immunosuppressive women, due to for example immunomodulating medications or HIV infection, are at increased risk of cervical dysplasia and cancer (10).

Studies have suggested that the vaginal microbiota can affect the risk of HPV infection, cervical dysplasia, and cancer. A non-lactobacillus-dominant vaginal flora was associated with higher prevalence of infection with both lr- and hr-HPV in comparison with a vaginal flora with dominance of lactobacillus species (65, 66).

In recent years, the impact of diet and nutritional factors has been studied. Some studies suggest that a higher intake of antioxidants (for example vitamins A, C, E, carotenoids, lycopene, lutein, cryptoxanthin) may reduce the risk of cervical dysplasia or cancer (67, 68). However, more evidence is needed to clearly state the role of nutritional factors in cervical carcinogenesis.

Histopathology

The two most common histopathological subtypes of cervical cancer are squamous cell carcinoma (SCC) representing 75-85% of all cases and adenocarcinoma (ADC) representing 10-20% of all cases. The remaining percentage consists of adenosquamous carcinoma and more rare forms of cervical cancer such as carcinoid tumors, neuroendocrine tumors, adenoid cystic carcinoma and malignant melanoma (49). Both SCC and ADC are strongly associated with HPV, HPV 16 is more common in SCC and HPV 18 is more often detected in ADC (69). For the rarer subtypes of cervical cancer, HPV DNA was detected in 72% of cases with neuroendocrine carcinoma and undifferentiated tumors in one study (70).

Cervical cancer screening has been very efficient in reducing cases of cervical cancer, especially SCC (71). However, in recent years the incidence of adenocarcinoma has been increasing in several countries (45, 71-73). In 2019, 29% (154/533) of all new cases of cervical cancer consisted of ADC in Sweden compared to 7% (57/773) in 1970 (41). Cytological screening has a lower sensitivity to detect ADC due to the precursors and disease originating from glandular epithelium in the cervical canal (74). But, in Sweden a nationwide audit showed that the national cervical screening program was effective in reducing the incidence of ADC (75).

Diagnosis and staging

Many cases of cervical cancer are detected through the cervical screening program. If cervical cancer is suspected through symptoms or abnormal screening results, the woman should undergo colposcopy with biopsies from the cervix, and endocervical curettage is recommended. If the biopsies are inadequate or normal but the suspicion of cancer remains high a diagnostic conization should be performed for more accurate assessment (76).

Staging of cervical cancer is based on clinical staging according to the International Federation of Gynecology and Obstetrics (FIGO). In 2018, FIGO updated the recommendations for staging because there has been progress in the use of imaging modalities in the staging of cervical cancer. The amendments to the staging classification included imaging and pathological assessment of the pelvis and potentially affected lymph nodes (77, 78). However, it is important to remember that approximately 84% of all cervical cancer cases are diagnosed in developing countries with limited medical resources (38). Therefore, the new amendments allow the clinician to stage the cancer according to available resources (77, 78). In Sweden, assessment of the stage is made by palpation during anesthesia combined with cystoscopy and sometimes proctoscopy and rectoscopy. Computer tomography or PET-CT of the thorax and abdomen in combination with magnetic resonance imaging of the pelvis is performed to assess tumor size, pelvic spread, lymphadenopathy, and distant metastases. Staging is determined depending on: invasion depth, invasion width, tumor size and tumor spread (49). A description of the different stages of cervical cancer according to the 2018 FIGO staging system is shown in Table 1 (77).

Table 1. FIGO staging for cervical cancer 2018. Adapted from Lee *et al.* (77)

2018 FIGO Staging System for Cervical Cancer	
Stage	**Description**
I	Carcinoma is strictly confined to the cervix
IA	Invasive carcinoma with maximum depth of invasion <5 mm
IA1	Stromal invasion <3 mm in depth
IA2	Stromal invasion ≥3 mm and <5 mm in depth
IB	Invasive carcinoma confined to the uterine cervix, with measured deepest invasion ≥5 mm
IB1	Tumor measures <2 cm in greates dimension
IB2	Tumor measures ≥2 cm and <4 cm in greatest dimension
IB3	Tumor measures ≥4 cm in greatest dimension
II	Carcinoma invades beyond the uterus, but has not extended onto the lower third of the vagina or to the pelvic wall
IIA	Limited to the upper two-thirds of the vagina without parametrial involvement
IIA1	Tumor measures <4 cm in greatest dimension
IIA2	Tumor measures ≥4 cm in greatest dimension
IIB	With parametrial involvement but not up to the pelvic wall
III	Carcinoma involved the lower third of the vagina and/or extends to the pelvic wall and/or causes hydronephrosis or nonfunctioning kidney and/or involves pelvic and/or para-aortic lymph nodes
IIIA	Involves the lower third of the vagina, with no extension to the pelvic wall
IIIB	Extension to the pelvic wall and/or hydronephrosis or nonfunctioning kidney from tumor
IIIC	Involvement of pelvic and/or para-aortic lymph nodes, irrespective of tumor size and extent
IIIC1	Pelvic lymph node metastasis only
IIIC2	Para-aortic lymph node metastasis
IV	Carcinoma has extended beyond the true pelvis or has involved the mucosa of the bladder or rectum
IVA	Spread to adjacent pelvic organs
IVB	Spread to distant organs

Treatment and prognosis

The primary treatment for early stages of cervical cancer is surgery. For microinvasive disease (stage IA1) without lymphovascular space invasion, a conization with negative margins or a simple hysterectomy is adequate as treatment. For stages IA2, IB1 and IIA1 radical hysterectomy and pelvic lymph node dissection is recommended as the standard treatment (79). The hysterectomy can be performed by laparotomy or by minimally invasive surgery (laparoscopy or robotic-assisted surgery). Previous studies have found superior results for robotic-assisted surgery compared to laparotomy and equal results for robotic-assisted surgery compared to laparoscopy (80). However, in 2018 a large randomized trial compared minimally invasive radical hysterectomy to open radical hysterectomy among women with cervical cancer stages IA1-IB1 with the aim of studying the disease-free survival rate. The study found lower disease-free survival and overall survival for women treated with minimally invasive radical hysterectomy (81). Though, in the Swedish quality registers no such differences have been seen, but further studies are necessary (82). For women who wish to preserve their fertility, radical trachelectomy can be considered if the tumor is ≤2 cm in size (79).

For cervical cancer stages IB2 and IIA2-IVA curative chemoradiotherapy is recommended as primary treatment since four articles reported improved survival with a combination of cisplatin and radiation in the treatment of patients with locally advanced disease in 1999 (83-86). Today the treatment is a combination of brachytherapy, external radiotherapy, and concomitant cisplatin (79). For disseminated disease, palliative chemotherapy to relieve symptoms and improve life quality is used (69). Regardless of stage, there is no difference in the treatment of SCC and ADC.

Several factors are suggested to be related to the prognosis of cervical cancer. Stage at diagnosis is one prognostic factor (87) and the presence of lymph node metastases has been shown to be of great importance (88). Furthermore, increasing age at diagnosis is associated with worse prognosis (42, 87). The impact of histopathological subtype regarding the prognosis has been debated. Some state that ADC has a worse prognosis compared to SCC (89) while some have found no difference in prognosis (87, 90). The 5-year relative survival of cervical cancer has increased in several countries during the last decades, although in the U.S. no increase in survival for metastatic disease has been found (42, 87, 91-93).

Cervical cancer screening

The organized cervical screening program in Sweden

All women, 23-64 years old, are included in the organized cervical screening program in Sweden. Women 23-49 years are invited every third year and women aged 50-64 years are invited every seventh year (every fifth year for regions using mRNA HPV testing). If no test is registered at the age of 64 years, the woman will be invited yearly until a normal test result has been registered or up to the age of 70. Since 2015, testing for hr-HPV has been recommended as primary screening method for all women $\geq$30 years in Sweden and it was implemented in the county of Skåne in January 2017. For women <30 years cytology is recommended due to a higher prevalence of non-persistent HPV infections at these ages. For women at 41 years of age testing for both HPV and cytology is recommended. In the case of abnormal screening test results, reflex testing with analysis of hr-HPV is used for women <30 years and cytological analysis for women $\geq$30 years. Women that have not attended the screening are reminded to do so yearly. If a woman has not attended for >3 years since her last invitation, the national recommendation is to remind her to do so through a telephone call. If a woman has not attended for >4 years since her last invitation, the woman should be offered an HPV self-sampling kit. In Sweden, all cervical screening is free of charge (94).

For a screening program to be effective, a high coverage is essential. In 2020, the national coverage was 80.3% (range 66.8-90.3%) in Sweden (95). This is the highest coverage level comparing back to 2012 and a very good coverage compared internationally, but it is below the national recommended coverage level of 85% (94). Studying coverage by age class, women in the age range of 23-30 years and 51-60 years reach a coverage level >85% while women aged 31-50 years and 61-70 years do not reach the goal, with the lowest coverage among women aged 61-70 years of 55.2% (96). One of the greatest risk factors of cervical cancer is non-attendance to the cervical screening program. A Swedish study found that 64% of cervical cancer cases and 83% of advances cases were diagnosed among screening non-attendees (75). Darlin *et al*. found that the four most common reasons for not attending cervical screening were "uncomfortable with vaginal examination", "feel healthy", "lack of time" and "experience of unfriendly health workers" (97).

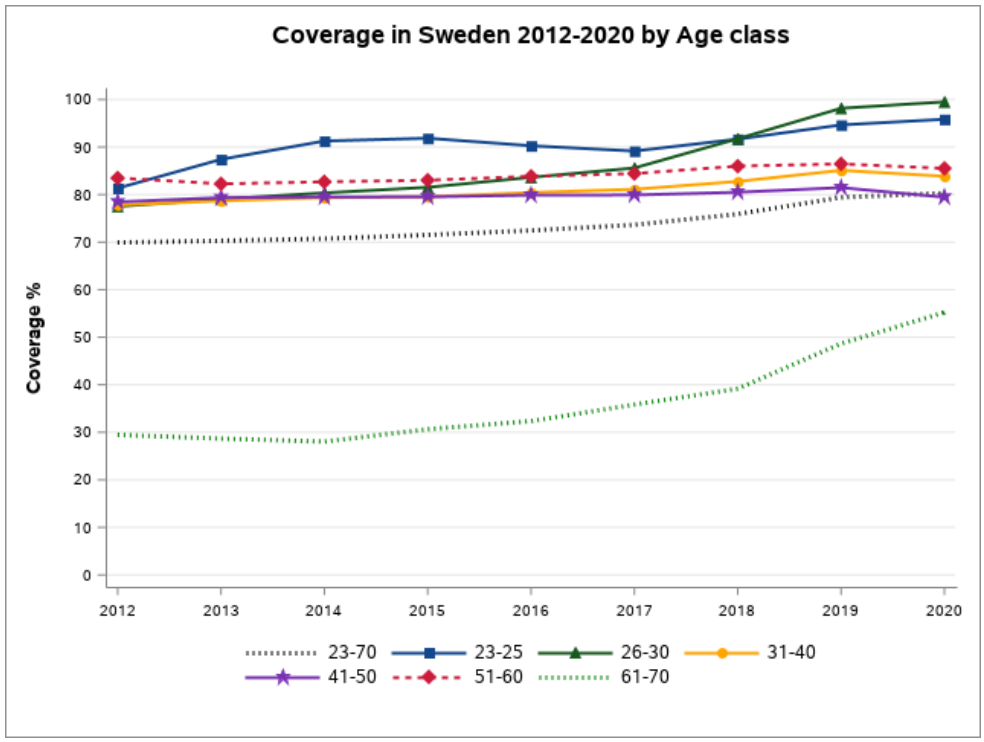

Figure 5. Coverage of the national cervical screening program in Sweden 2012-2020 by age class. Source: Swedish National Cervical Screening Registry (NKCx) (96).

Cytology

Since the beginning of cervical screening, cytological assessment has been used to find cervical dysplasia or cancer. The Papanicolaou test (Pap test) was used until

the late 2000s when liquid-based cytology (LBC) was introduced. LBC has a lower rate of unsatisfactory samples compared to the Pap test (98-101) and has the advantage that additional analyses can be performed without taking a new test (reflex testing) (49). A large Swedish study reported an improved detection rate of high-grade cervical dysplasia for LBC (98), while a meta-analysis found equal sensitivity and specificity in detecting high-grade cervical dysplasia for LBC and the Pap test (102). The sensitivity and specificity for LBC in finding high-grade dysplasia was reported as 57.1% and 97.0% respectively in the meta-analysis (102). Even though an increased sensitivity is desirable, cytological assessment as a tool to discover cervical dysplasia has prevented many cases of cervical cancer across the globe for many years. However, among older women, cytology is a less sensitive diagnostic tool. In a study of women 50 years or older the efficiency of cytology was only 20% compared to cytological assessments at ages 30-34 years (103). Several other studies have supporting results showing a lower sensitivity for cytology among older women (104-107). With increasing age, the TZ migrates upward into the cervical canal, creating difficulties in acquiring an adequate cervical sample, resulting in lower sensitivity for cytology. A different diagnostic tool should be recommended for older women. Cytological changes in the cervix are described according to the Bethesda system (52).

HPV testing

Since the discovery of hr-HPV as a near to necessary component of the carcinogenesis of cervical cancer (108), researches has tried to find methods to further improve cervical cancer screening. The first studies on HPV testing within clinical settings were published in the early 1990s (109). Subsequently, after numerous studies, HPV testing has become widely used and is one of the cornerstones of cervical cancer screening. HPV-based screening provides 60-70% better protection against cervical cancer compared to cytology-based screening and screening intervals can be extended to at least five years. Because of the improved sensitivity and better long-term protection, HPV-based screening is recommended for all women aged 30 years and older (110).

There are more than 250 distinct commercial tests available for detection of hr-HPV, of which seven are approved by the U.S. Food and Drug Administration (FDA) (111). There are two distinct groups of HPV assays: DNA- and mRNA assays. HPV samples analyzed with a DNA assay have shown a significantly higher sensitivity for detection of CIN2 compared to cytology but with the expense of a slightly lower specificity due to identification of transient HPV infections (112). HPV samples analyzed with an mRNA assay have a similar sensitivity to HPV DNA samples but with improved specificity (113-115). Several studies have found that the DNA and mRNA assays on HPV samples are equally suitable for cervical screening (116, 117). In a large Swedish study by Forslund *et al.*, a cohort of >95,000 women were

followed for up to seven years and the longitudinal sensitivity of HPV DNA and mRNA cervical samples were calculated. The study found comparable results from the two assays and the authors concluded that both assays can be used for cervical screening of women >30 years with screening intervals of 5-7 years (118).

One of the most common mRNA tests in use is the FDA-approved Aptima test. The Aptima assay detects 14 hr-HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 (116). The mRNA assay is suggested to be superior in detecting relevant hr-HPV infections in comparison to a DNA assay. Since most people with hr-HPV infection do not develop precancerous lesions or cervical cancer, it is important to find a diagnostic tool that can identify individuals with a risk of development of precancerous lesions. HPV DNA assays can only detect the presence or absence of the HPV virus. The HPV mRNA assay detects the oncogenes E6 and E7 which are necessary factors in the development and maintenance of precancerous lesions and cervical cancer (7, 9) suggesting the mRNA assay to be more specific in finding high-grade dysplasia. Several studies have found evidence that women positive for HPV mRNA had an increased probability of developing lesion progression (119-121). However, this also means that an HPV DNA sample can show the presence of HPV and an HPV mRNA sample taken at the same time can be negative. Forslund *et al*. found a lower sensitivity for the mRNA assay compared to the DNA assay among women with normal cytology <30 years of age, this might be the result of a higher prevalence of transient infections among younger women (118). The protective effect of a negative Aptima HPV mRNA sample has been proven to be equal to a negative HPV DNA sample in the long-term risk of CIN 2 or higher (122). A limitation of the Aptima mRNA assay is that there is no internal control for human genes. In several DNA assays there is an internal control for human genes which confirms sample adequacy and decreases the risk of false negative results (117).

Self-sampling

When HPV tests were introduced to cervical screening, a new potential opportunity for cervical screening was presented in terms of HPV self-sampling tests. Self-sampling tests can be taken by the woman herself at a time and place that suits her, which could be a way to reach screening non-attending women. With self-sampling, the obstacles of fear of gynecological examination, lack of time and previous experience of unfriendly health personnel would be avoided. In the current national guidelines in Sweden, vaginal HPV self-sampling is recommended for screening of long-term screening non-attending women (94). However, as of September 2020, only seven out of 21 counties in Sweden had implemented this recommendation (123).

HPV self-sampling tests can be conducted by a vaginal sample or a urinary sample. Both HPV DNA- and mRNA assays are available as self-tests. In a large meta-analysis from 2018, the absolute sensitivity in vaginal HPV self-samples to detect

CIN2 or worse in a screening setting was 77% for hr-HPV assays based on signal amplification and 96% for hr-HPV assays based on polymerase chain reactions. The corresponding pooled specificity to exclude CIN2+ was 84% and 79% respectively. In comparison with cervical clinician HPV sampling, vaginal self-samples analyzed with hr-HPV assays based on signal amplification were less sensitive and hr-HPV assays based on polymerase chain reactions were equally sensitive but less specific (124). Other studies have found comparable results of HPV vaginal self-sampling and clinician sampling in referral populations (125-127) and a recent Swedish study found similar results of HPV vaginal self-sampling and clinician sampling in a screening setting (128). Vaginal self-sampling with Aptima mRNA assay has been reported to be less sensitive but equally specific to clinician-taken samples in detecting CIN2+ (129). However, in a recent randomized controlled trial, vaginal self-sampling with Aptima assay was found to detect a comparable proportion of high-grade dysplasia compared to regular screening (130). Sanner *et al*. investigated the possible effects of the menstrual cycle on vaginal self-sampling and found that there was a consistency in the self-sampling HPV results throughout the menstrual cycle (131). Vaginal self-sampling is generally accepted and preferred over clinician sampling among women of various ages as cervical screening method. Vaginal self-sampling is reported to be easy to use, convenient, private, less painful and discomforting, and less embarrassing. But, among many women preferring vaginal self-sampling, an insecurity about if the specimen was correctly collected was reported. Clinician sampling was associated with greater confidence that the specimen was properly collected (132).

Urinary sampling as an HPV self-sampling method has been shown to be accepted by women and it has been reported that women feel more confident in that they have correctly performed the urinary sample compared to a vaginal self-sample (133, 134). There are several studies investigating the use of urinary samples as HPV self-sampling tests, with varying results. Most studies have reported that HPV DNA-based urine testing is concordant with cervical HPV DNA samples and has a high sensitivity of 83-100% in detecting high-grade dysplasia (126, 133-135). However, some studies have found a lower sensitivity of HPV DNA-based urine testing (136-138). The number of studies investigating the sensitivity of an HPV mRNA-based urine test is very limited. One study from Padhy *et al*. found a low sensitivity of 45.5% in detecting CIN2 or worse for urinary HPV mRNA testing with Aptima assay, but a higher specificity of 75.0% (139).

As previously mentioned, HPV self-sampling has the possibility of increasing the attendance to cervical screening among never- or under-screened women. In a large meta-analysis, the response rate for vaginal self-sampling among under-screened women was on average 19.2% (124). Previous studies from Sweden found a response to vaginal self-sampling of 15-58% among women aged 30-65 years of age who had not attended cervical screening for 6-9 years (97, 140-144). In a Danish study, higher detection rates of CIN2+ were found with HPV self-samples offered

to non-attendees in comparison with cytology-based screening (145). Reasons for not returning a self-test have been explored by a few studies through questionnaires, with the most common reasons being: opportunistic screening outside the screening program, preference of a regular screening procedure, low confidence in collecting the specimen correctly, anxiety, physically uncomfortable, an opinion that screening is unnecessary and pregnancy/previous labor (146-148).

If a woman is testing positive for hr-HPV in a self-sample, it is important to remember that she needs to be invited to a follow-up examination including cervical testing for renewed HPV analysis and/or cytological assessment and/or direct referral to colposcopy. A high compliance to follow-up is essential if self-sampling is to be used as a screening method. Previous studies have found a compliance to follow up of 70-100% among women aged 30-65 years with no cervical sample for 5.5-9 years (97, 140-142, 149-151).

Follow-up of screening results

The purpose of cervical cancer screening is to find and treat precancerous lesions to prevent cervical cancer. If a woman is persistently hr-HPV-positive or precancerous lesions are detected through screening, the woman is referred to colposcopy. Colposcopy is a visual inspection of the cervix and the TZ with help of a magnified and illuminated view from a colposcope. Acetic acid solution and iodine solution is applied to improve areas with abnormal epithelium. The colposcopy findings are classified based on the Swede Score, including assessment of uptake of acetic acid, margins and surface, capillary vessels, lesion size and iodine staining. Each category of the Swede Score is scored from 0-2 and added together to a score between 0 and 10 (152). Depending on the Swede Score, HPV status, cytological abnormalities, age, and risk factors it is decided whether cervical biopsies are indicated. A score of 0-4 represents a low risk of high-grade dysplasia. For a score $\geq$8 treatment without previous biopsy is recommended due to a high risk of high-grade dysplasia (152). Histopathological abnormalities of the cervix are described according to the two-tiered nomenclature recommended by WHO (153). Colposcopy is a subjective examination and the sensitivity and specificity of colposcopy in finding high-grade lesions are highly variable in different studies, mainly because two different methods are used of assessing the output of colposcopy: the outcome based upon the colposcopic impression that high-grade dysplasia (CIN2+) is present, and the outcome based upon taking a biopsy because there is thought to be some disease present (154). The weighted mean sensitivity and specificity in detecting CIN2+ for 13 studies where both methods were available were 75.1% and 71.0% respectively (154). More than one biopsy is associated with a higher sensitivity in detecting high-grade lesions (155).

Among older women, the TZ is transferred into the cervical canal and might not be visualized during colposcopy (49). The TZ is categorized into types 1-3. TZ type 1

is completely visible at the ectocervix, TZ type 2 is partly located at the endocervix but the SCJ is completely visible, and TZ type 3 is partly located at the endocervix and the SCJ is not visible (94). TZ type 3 is common among postmenopausal women (104). When the TZ is not visible, there will not be representative biopsies during colposcopy. Among these women a diagnostic excision of the cervix is an option. In a Swedish study, 15% of women >40 years old with persistent hr-HPV and normal cytology were diagnosed with histopathological CIN2+ through a diagnostic loop electrosurgical excision procedure (LEEP) (156) indicating the need to consider additional diagnostic procedures in this patient category.

Treatment of precancerous lesions

Not all precancerous lesions need treatment. For histopathologically detected LSIL, active expectancy with colposcopy, HPV testing and cytological analysis is the recommended alternative in Sweden (94). The majority of LSIL cases regress spontaneously and the risk of development to cervical cancer is low (157, 158). However, for histopathologically detected HSIL the standard procedure is excisional treatment since there is an increased risk of development to cervical cancer (55, 94). There are two principal different treatment options for HSIL; ablative management (cryotherapy, thermal coagulation, and laser ablation) or excisional treatment (LEEP, laser conization and cold knife conization). Both treatment options are associated with a risk of complication of bleeding, infection and cervical stenosis (159). Excisional treatment has the advantage of generating a histopathological diagnosis and is most used in Sweden. However, in a meta-analysis, ablative and excisional treatment were reported to be similar in eradicating dysplasia and in the risk of future invasive cancer following treatment, although the meta-analysis highlights the need for improved studies on the subject (160).

An exception regarding treatment of HSIL are women under the age of 25 with histopathologically detected CIN2+, in which cases active expectancy with colposcopy, HPV testing and cytological analysis is recommended. This recommendation is based on the fact that the risk of development from CIN2+ to cervical cancer is low among women <25-30 years of age (94, 161). Furthermore, treatment of precancerous lesions can increase the risk of late miscarriage and premature delivery in case of pregnancy. In a large study from Denmark, the risk of premature delivery increased by 6% per each extra millimeter of cervical excision with LEEP over 12 mm (162). For excisions under 10 mm the risk of premature delivery is considered very small (163). The results of a meta-analysis reported a significantly higher rate of miscarriage in the second trimester among women treated for precancerous lesions (studies with both excisional and ablative treatment included in the study) (164). Cervical stenosis is another possible adverse event after treatment of precancerous lesions. Some have found an elevated risk of cervical

stenosis among older women, while others state the height or volume of tissue to be important predictors of stenosis (165-167).

HPV vaccination

In 2006, vaccination against HPV was introduced in Sweden and in 2012 it was implemented in the Swedish childhood vaccination program for all girls in grade 5 or 6 (94). Since the autumn of 2020, HPV vaccination has been offered to both girls and boys in grade 5 (168). The coverage of two doses of vaccine for girls born in 2007 was 82.9% (168). There are three available HPV vaccinations: The 2-valent vaccine against HPV 16 and 18, the 4-valent vaccine against HPV 6, 11, 16 and 18 and the 9-valent vaccine against HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 (94). The HPV vaccines are considered safe since they have no known major side effects (169). A meta-analysis reported 68% decrease of infection with HPV 16 and 18 comparing pre-vaccination and post-vaccination. A cross-protection with reduction of infection with HPV 31, 33 and 45 was also reported (170). HPV vaccinations has been shown to substantially reduce the risk of high-grade dysplasia and invasive cervical cancer (171, 172). A significant decrease in anogenital warts was reported in girls aged 13-19 years. A reduction in anogenital warts was also seen among boys <20 years of age and women 20-39 years of age, indicating herd immunity (170). A Nordic study showed a long protection of at least 10 years after vaccination with the 4-valent HPV vaccine (173). However, it is still important to encourage women to attend to the cervical screening since not all hr-HPV types are included in the vaccine. In Sweden, studies have reported a higher or equal attendance rate at cervical screening among vaccinated women compared to unvaccinated women (174, 175). As of June 2020, 107 countries had implemented a national vaccination program for HPV, which is very positive (176).

Aims of the thesis

The overall aim of the thesis was to analyze if the organized cervical screening program in Sweden can be improved by using vaginal self-collected samples and an HPV mRNA assay, with the main focus on screening non-attendees. The aim was also to obtain knowledge if cervical cancer incidence and survival has changed since the implementation of a screening program in the late 1960s.

The main hypothesis was that the use of vaginal self-collected HPV samples would increase the attendance at cervical screening among long-term screening non-attendees. Furthermore, the hypothesis was that the survival of cervical cancer had improved in all ages since the 1960s.

Specific aims

Paper I

To investigate time trends for incidence and long-term net survival in the morphologic subtypes and stages of cervical cancer in Sweden during the period 1960 to 2014 using data from the Swedish Cancer Registry (SCR).

Paper II

To investigate the sensitivity and specificity of the Aptima HPV mRNA assay in detecting HSIL, AIS or cervical cancer in vaginal self-samples as well as from urine in comparison with clinician-collected cervical HPV samples.

Paper III

To analyze the response rate of a free of charge offered vaginal HPV self-sample sent to home as well as the HPV mRNA prevalence among women 69-70 years of age.

Paper IV

To investigate the response rate of a free of charge self-collected vaginal hr-HPV sample, sent to women in 2017, who had not attended organized cervical cancer screening for ≥7 years; to study the compliance with follow-up among women positive for hr-HPV in the self-collected vaginal sample; to analyze the prevalence of severe cervical dysplasia (HSIL, ASC-H, AIS) or cancer among the responders; and to explore, by telephone interviews, the reasons for not returning a self-collected vaginal hr-HPV sample.

Paper V

To investigate the response rate of a free of charge self-collected vaginal hr-HPV sample, sent to women in 2018, who had not provided a cervical smear for ≥7 years; to explore the attendance rate at follow-up among women positive for HPV in the self-collected vaginal sample; and to analyze the prevalence of hr-HPV and severe cervical dysplasia or cancer among the responders. Furthermore, the study aimed to investigate the distribution of responses and HPV positivity among different age categories.

Material and methods

Paper I

In this population-based register study, the incidence and net survival of cervical cancer in Sweden from 1960 to 2014 was analyzed using the SCR.

The Swedish Cancer Registry (SCR)

In 1958, the population-based nationwide SCR started registration. The register contains information about all patients with premalignant and malignant conditions and certain benign tumors (177). The completeness of the SCR is over 95% and the high coverage of the registry is secured by the compulsory task for clinicians, pathologists, and cytologists to independently register above patients in the register (178). The SCR receives data once yearly from six regional registries. Of the cancer cases, 98% are verified by morphology (177). Follow-up of the patients in the SCR is close to complete up to time of death or emigration because of the identification number system in Sweden (179).

Cohort

During the period 1960-2014, 230,146 women with cervical tumors were identified in the SCR. The women were matched with the Swedish Death Registry up until May 7th, 2020. After applying the exclusion criterion listed in Figure 6, 29,579 cases of invasive cervical cancer were included in the study. For the analyses the women were grouped according to morphology (SCC and ADC), age at diagnosis (18-44, 45-54, 55-64, 65-74 and $\geq$75 years) and stage (I-II and III-IV).

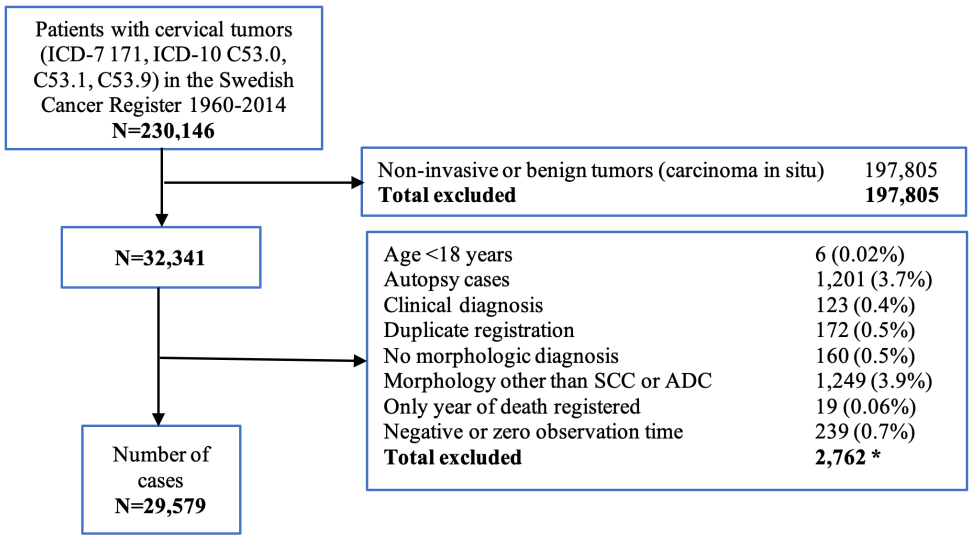

Figure 6. Flow chart of included and excluded cases of cervical tumors in the study.
ICD-7 171 tumor of cervix uteri. ICD-10 C53.0 malignant tumor of the endocervix, C53.1 malignant tumor of the exocervix, C53.9 unspecified localization of malignant tumor of the cervix uteri.
*Some cases fulfilled more than one exclusion criteria.

Statistical analyses

Incidence rates were age-standardized to the World Standard population 2011 (180). Survival time was measured from date of diagnosis until date of death, date of emigration, or May 7, 2020. Net survival was estimated in a relative survival framework, which is the standard approach for population-based studies of cancer patient survival (181).

Flexible parametric models were used to estimate net survival (182). Expected mortality rates for women, stratified by age and calendar year, were retrieved from the Human Mortality Database (http://www.mortality.org) based on data from Statistics Sweden. The model included the main effects of age at diagnosis, morphology, and year of diagnosis as restricted cubic spline with three degrees of freedom. The baseline cumulative excess hazard was modeled as restricted cubic spline with five degrees of freedom and the time-varying effects were modelled using two degrees of freedom. Temporal trends in net survival within each age group along with age-standardized net survival were estimated based on this model using the International Cancer Survival Standard (ICSS) population number 2 (183). An illustration of the analytic process is available at https://www.pauldickman.com/software/stata/age-standardise-standsurv/. Stata version 16 (StataCorp, TX, USA) was used for the statistical analyses.

Paper II

Paper II was a diagnostic accuracy cross-sectional study to investigate the sensitivity and specificity of vaginal- and urine self-samples compared to cervical samples analyzed by Aptima HPV mRNA assay.

Study population

Women referred to colposcopy due to abnormal findings in their cervical screening results or for monitoring of dysplasia following previous excisional treatment were used as the study population. Between February 2015 and November 2016, 216 referral patients attending the women's clinic in Lund gave their written consent to participate in the study. Exclusion criteria were previous hysterectomy, history of gynecological cancer, or current oncological treatment. Seven patients were excluded due to previous hysterectomy or absence of an HPV, cytology, or histopathological sample. Written and oral instructions for the self-sampling procedures were given to all participating women.

Urinary self-sampling

The urinary HPV sample was the first sample to be obtained in the study. The women were instructed to leave 10-50 mL of first stream urine in a plastic container. Within 10 minutes the participant or the assisting nurse transferred 2 mL by a pipette into a test tube containing transport media (Aptima Urine Specimen Collection Kit Urine Specimens, Hologic Inc., MA, USA).

Vaginal self-sampling

The vaginal HPV self-sample was the second sample to be obtained in the study. The women were instructed to collect the self-sample by placing a cotton swab (Aptima Vaginal Swab Specimen Collection Kit, Hologic Inc., MA, USA) 3-4 cm up into the vagina and rotating it. Thereafter, the cotton swab was put into a tube containing transport media.

Clinician performed examination

After the self-samples were collected, a gynecological examination was performed. A clinician-taken HPV sample from the cervix was collected with a swab (Aptima Vaginal Swab Specimen Collection Kit, Hologic Inc., MA, USA) and a cervical sample for cytological assessment was obtained with the ThinPrep device (PreservCyt Solution, Hologic Inc., MA, USA). Colposcopy was then performed. If there was an indication based on the clinical situation or the findings of colposcopy, a histopathological specimen was obtained through a cervical biopsy or through LEEP.

Analysis of collected specimens

The HPV samples were analyzed using the Aptima HPV mRNA assay on a Panther instrument according to the manufacturer's instructions at the Laboratory Medicine, Lund. The Aptima HPV assay detects 14 hr-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) (116). The cervical specimens for cytological assessment were assessed as routine LBC samples at the Department of Clinical Cytology, Lund and graded according to the Bethesda system (52). The histopathological specimens were assessed at the Pathology Department, Lund and classified according to the two-tiered nomenclature recommended by WHO (153).

For comparative analyses of the HPV tests with cytology and histopathological specimens, the results of the histopathological assessment were used.

Statistical analyses

Statistical comparisons were based on the binomial distribution and 95% confidence intervals (CI) were given. Comparisons among the HPV results of clinician-taken cervical samples, vaginal self-samples and urinary self-samples were conducted using Pearson and McNemar's Chi-Square test. The Spearman rank-order correlation coefficient measured the association between the variables. All comparisons were two-sided and p-values less than 0.05 were considered statistically significant.

To achieve a power of 90% at a significance level of $\alpha = 0.05$ a minimum of 188 patients with HPV infections are required to detect a difference of at least 10% when the expected sensitivity for test 1 (HPV self-sample) is 0.95 and for test 2 (clinician-taken cervical HPV sample) is 0.85.

The statistical analyses were performed using IBM SPSS statistics version 24 and MedCalc Software©.

Paper III-V

In paper III-V the response rate to a vaginal self-collected HPV sample was analyzed among long-term screening non-attendees or women in the upper age screening limit in Sweden. The southern regional cervical screening registry was used to identify women with a home address in the county of Skåne who had not provided a cervical sample according to the screening guidelines. The southern regional cervical screening registry encompasses information on all smears, organized and spontaneously taken, in the region.

Self-sampling

In the county of Skåne, the Aptima HPV mRNA assay (Hologic, San Diego, CA, USA) is used for analysis of all HPV samples taken in the region, whether clinician-taken or self-sampled. The Aptima HPV assay detects 14 hr-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) (116). The analysis was carried out on a Panther instrument, according to the manufacturer's instructions at the Laboratory Medicine, Region Skåne, Lund.

For the self-sampling procedure, a self-sampling kit, free of charge, was sent to the home address of the women. The kit contained: 1) information about cervical screening and hr-HPV infection and written and illustrative descriptions on how to perform the self-sampling, 2) one Aptima Multitest Swab and a tube prefilled with 2.9 ml Aptima Multitest Swab Transport Media (Hologic Inc., MA, USA), 3) one cylindrical container for transportation of the self-sample, 4) pre-printed labels with each woman's social security number to mark the test, and 5) one prepaid padded return envelope addressed to the Laboratory Medicine, Region Skåne, Lund.

The women were instructed to collect the self-sample by placing a cotton swab 3-5 cm up into the vagina and rotating it, then placing the cotton swab into the tube containing the transport media. The women were asked to mark the tube with the pre-printed labels with their social security number, which were used for identification during the analytical process. Finally, the test tube was put into the transportation tube and sent through the regular mail using the return envelope.

Study procedure

Information about age group, number of women included in each study, time since last provided cervical sample, home address, date of dispatch of the self-sampling kit, date of inclusion of returned self-sampling tests and follow-up time for paper III-V is given in Table 2. No reminder letter was sent out for women who did not return the self-sample test.

Table 2. Detail information of the study procedure for paper III-V.

	Paper III	**Paper IV**	**Paper V**
Age	69-70 years	30-70 years	30-70 years
Number	1,000	6,023	19,766
Last provided cervical sample	≥5 years	≥7 years	≥7 years
Home address	Municipality of Lund	County of Skåne	County of Skåne
Date of dispatch	April 25, 2017	November, December 2017	May 22 and 28, 2018
Inclusion of tests until	June 10, 2017	May 31, 2018	May 31, 2019
Follow-up time	≈12-14 months	8-14 months	4-17 months
Reminder	No	No	No

Follow-up algorithm

Women with hr-HPV negative results were informed by an automatically generated letter from the Department of Laboratory Medicine and returned to the organized cervical screening. In the case of an invalid test result, the women were asked, through a letter, to contact a midwife station to make an appointment to provide a cervical HPV sample. Women with hr-HPV-positive results received a letter from the nearest midwife station with information about the presence of hr-HPV and an invitation to a clinical follow-up examination by the midwife within three months. The follow-up examination included a cervical sample for cytological analysis and HPV Aptima mRNA analysis. If the woman did not attend her follow-up examination, a reminder letter was sent. If no information about attendance at a follow-up examination was registered one year after the self-sampling kit was sent out, a second reminder letter was sent to women positive in the self-sample. In paper IV, this reminder was initially given by telephone and then by a letter to those who could not be reached by telephone. In the case of abnormal results at the follow-up examination, the women were managed according to current guidelines (94). In all studies, abnormal cytological findings were classified according to the Bethesda system (52) and abnormal histopathological findings according to the two-tier nomenclature recommended by WHO (153). In the case of several diagnoses, the worst diagnosis was used for the study.

Telephone interviews

In paper IV, reasons for not retuning a vaginal HPV self-sample were investigated through telephone interviews. In October 2018, 235 women that had not returned the self-sample test were randomly selected and called on the telephone number given to their care provider. Each woman was called a total of three times at different times of the day if not reached. Women that were reached were informed about the voluntary participation in the study. If the woman agreed to participate, she was asked the following question “Have you received a vaginal hr-HPV self-sampling invitation?”. If the answer was “no”, no further questions were asked. If the answer was “yes”, the following open question was asked “Why did you not perform the self-sampling?”. The answers were classified into five categories of reasons for not returning the self-sample: 1) emotional/attitude, 2) practical, 3) physical, 4) needless, or 5) other.

Statistical analyses

Statistical comparisons were based on the binomial distribution and 95% CI were given. Comparisons were made using a Pearson chi-square test. The comparison was two-sided and p-values less than 0.05 were considered statistically significant. Microsoft® Excel, Version 15.30 and IBM SPSS statistics version 26 were used on a Mac computer for the statistical calculations.

Ethical considerations

The ethical principles of a physician say to do no harm, to do good, to be justice and to respect the autonomy of the patient. The Helsinki declaration states the ethical principles for medical research. In the general principles it is stated that a physician shall act in the patient's best interests, a physician shall promote and protect patients' health, well-being and rights, and the ethical standards of the research shall ensure respect and protection for all human subjects (184). For the women participating in the studies included in this thesis, I believe that these aspects were met.

Paper I was a retrospective register study where previously collected data was used and no new data was collected. No patient consent was required. The benefit of the study was considered to be greater than the potential harm to the patients. The outcome of the study did not have any impact on the treatment or prognosis of the patients included in the study. However, the results of the study are expected to provide a gain in knowledge which might improve the diagnosis, treatment, and prognosis of cervical cancer patients in the future.

The women participating in study II gave their written consent to participate after receiving detailed information about the study. Participation was voluntary. The individual medical care of the women was not affected by study participation and all results were presented at group level.

In paper II, self-sampling was performed at the women's clinic. However, when using self-samples sent to a woman's home there are several ethical dilemmas that need to be considered. Firstly, the integrity and safety of each woman needs to be discussed. It is important to ensure that all women with an indication to take an HPV self-sampling test, receive the sent out self-sampling kit. There is a risk of the parcel getting lost in the post or being delivered to the wrong address. Not all women are aware of the screening intervals in cervical screening and when it is time for them to provide a new cervical sample. If the self-sampling kit is not delivered to the intended woman, she might not know that it is time for her cervical screening. However, yearly reminders of cervical screening are sent out. Sending the woman a cervical screening notification through a digital mailbox, SMS or a letter could also be a solution. Another solution is that the woman orders the self-sampling kit herself. It is also important to ensure that the right woman is taking the sent out self-sample. This is ensured by the parcel containing labels with the woman's social

security number. However, if the kit is delivered to the wrong address another person will receive the social security number, making it difficult to guarantee the personal integrity of each woman. However, since information about the woman's address is collected from the population register, which is updated on a daily basis, the risk of this is considered to be low. An alternative solution is to use a self-sampling device with an embedded chip with linkage between the identity of the woman and the self-sampling device which is used in parts of Denmark (185). Furthermore, when a woman has performed the self-sampling test it is of crucial importance that the parcel is delivered to the laboratory responsible for the analysis. It should be in the woman's self-interest that she receives a test result from her screening, and if not, that she contacts health care.

Another ethical dilemma to consider is the removal of personal contact that screening with self-sampling entails. Today, cervical screening is an opportunity to inform each woman about HPV infection and the importance of cervical screening. It is also an opportunity for the woman to ask gynecological questions or questions regarding sexual activities. This personal contact will not naturally occur when self-sampling tests are used. It is important that the information about hr-HPV in the self-sampling kit is clear and available in several languages. It is also important to spread the knowledge of cervical screening in the society to further increase the attendance rate to cervical screening. If a woman does not feel comfortable with the self-sampling procedure the opportunity for cervical HPV testing at a midwife station should still be available.

Even though there are some ethical issues to consider regarding self-sampling, the benefits of self-sampling are many. The woman can take the test at a time and place that is best for her. She does not need to take time off from work for the cervical screening, which is a benefit for society. Midwives can have more time for other health care work when midwife-collected cervical sampling decreases. In addition, self-sampling is cheaper for health care compared to midwife-collected cervical sampling (186).

In paper III-V no consent was collected from the participating women since offering self-collected HPV samples to long-term non-attendees is part of the national cervical screening program. Returning the self-sample was defined as the woman's consent to participate in the study. All cervical samples taken in Sweden are stored at a biobank (the biobank law 2002:297) and can be used for research if there is an ethical approval. Furthermore, results from the cervical screening in Sweden are stored at the Swedish National Cervical Screening Registry (NKCx). Each woman can voluntarily decide if she does not want her cervical sample stored and results from cervical screening saved.

Paper I (DNR 2015/789), paper II-IV (DNR 2013/390) and paper V (DNR 2013/390, amendment to DNR 2018/466) were approved by the Regional Ethical Review Board, Lund.

Results

Paper I

Women aged 18-44 years was the most common age group at diagnosis during the study and became more common in 2010-2014 (1960-69 37.1% vs 2010-14 46.2%, p<0.001). The oldest age group (≥75 years) was the least common age group for diagnosis of cervical cancer in 1960-69, but became more common after 1980 and forward.

The age-standardized incidence of SCC decreased until the year 2000, after which a stagnation in incidence was found and in 2014 a small increase was detected. The incidence of ADC continuously increased during the study (Figure 7). Since 1960, the proportion of diagnosed ADC cases in Sweden increased by almost a quarter (1960-69 6.0% vs. 2010-14 23.1%, p<0.001).

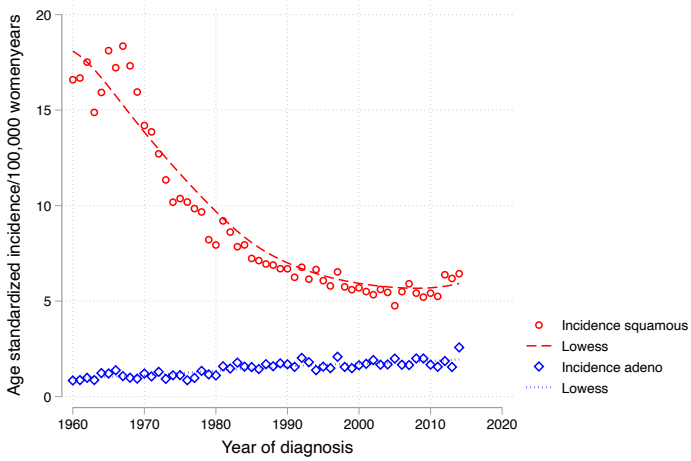

Figure 7. Age-standardized incidence depending on the morphology squamous cell carcinoma and adenocarcinoma per 100,000 women years standardized to the world population.

There was an increase in age-standardized 5-year net survival from 1960-2014 for SCC and ADC (Figure 8). The age-standardized 5-year net survival was slightly

higher for SCC compared to ADC, but the difference was very small and between 1990-2000 and 2010-2014 the difference was not statistically significant.

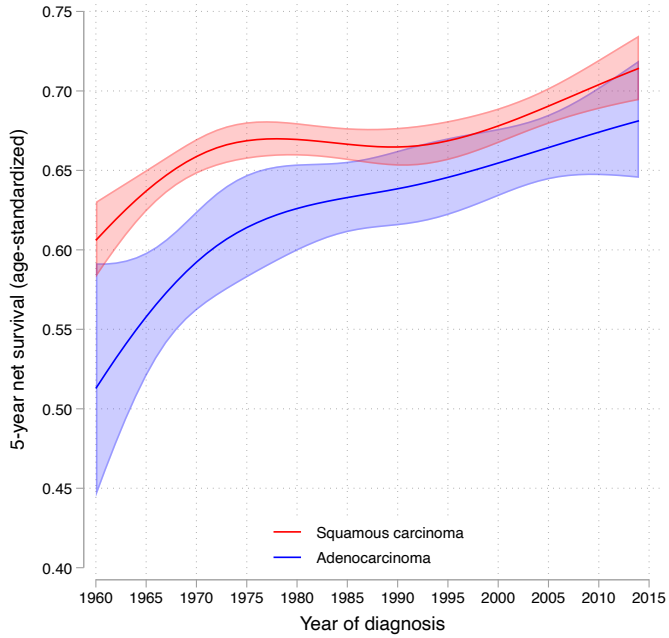

Figure 8. Age-standardized 5-year net survival for squamous cell carcinoma and adenocarcinoma with 95% confidence intervals.

Decreasing long- and short-term net survival with increasing age was detected for SCC and ADC. SCC demonstrated improved 1-, 5- and 10-year net survival for women aged 18-64 years since 1960 but decreased 5- and 10-year net survival for women ≥75 years. ADC demonstrated improved 1-, 5-, and 10-year net survival for all ages in the period 1960-2014 except for 5- and 10-year net survival for women ≥75 years (Figure 9).

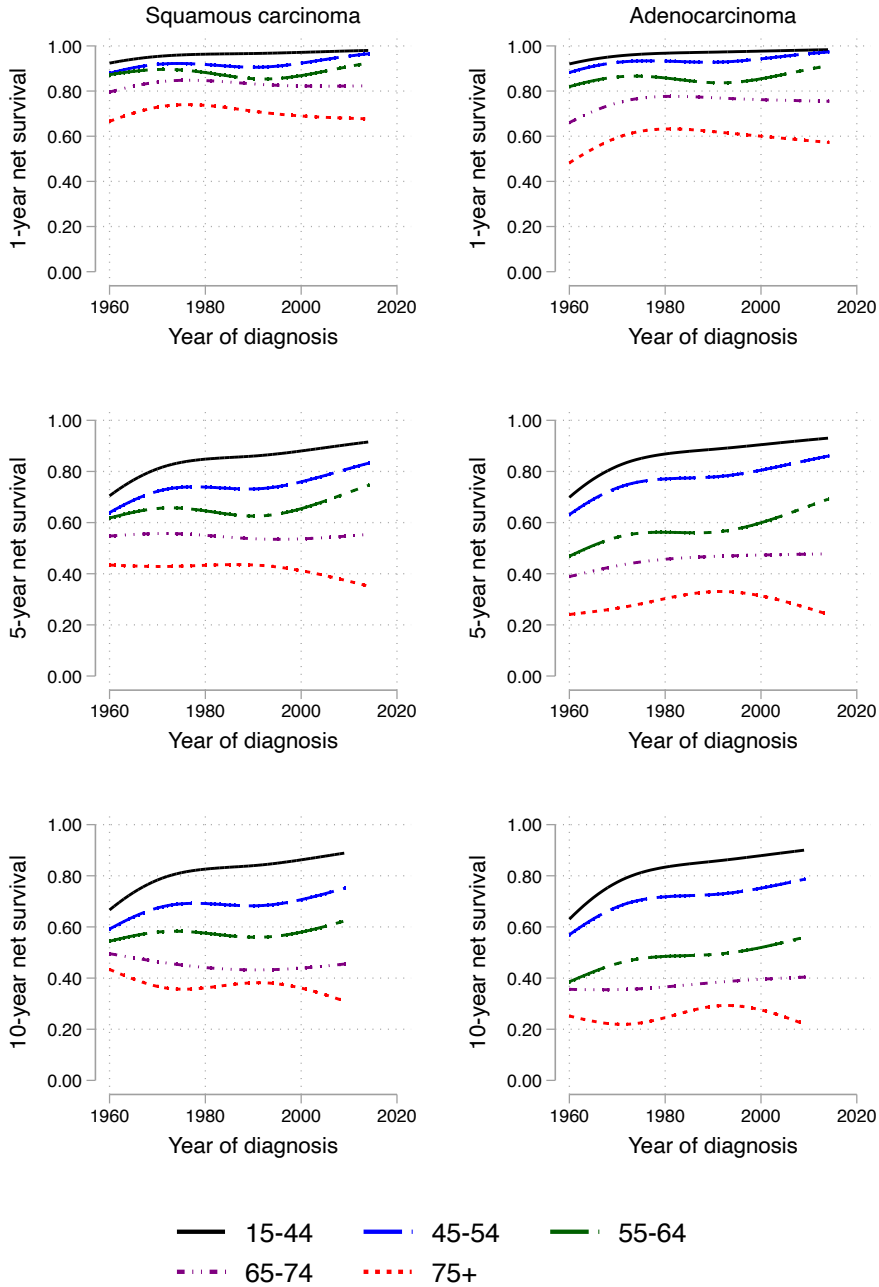

Figure 9. Time trends for 1-, 5-, and 10-year net survival according to age groups and for squamous cell carcinoma and adenocarcinoma.

Higher stage at diagnosis was associated with a worse net survival. Stable or increased 5-year net survival for stages I-II was demonstrated for SCC and ADC between 2005 and 2014 and there was no statistically significant difference in net survival between the morphologies in 2005-2009 and 2012-2014. The 5-year net survival for stages III-IV was improved for SCC and stable for ADC during 2005-2014. In 2005 there was no difference in 5-year net survival between SCC and ADC in stages III-IV; from 2006 to 2014, SCC demonstrated a better 5-year net survival than ADC but since 2011 the difference was not statistically significant (Figure 10).

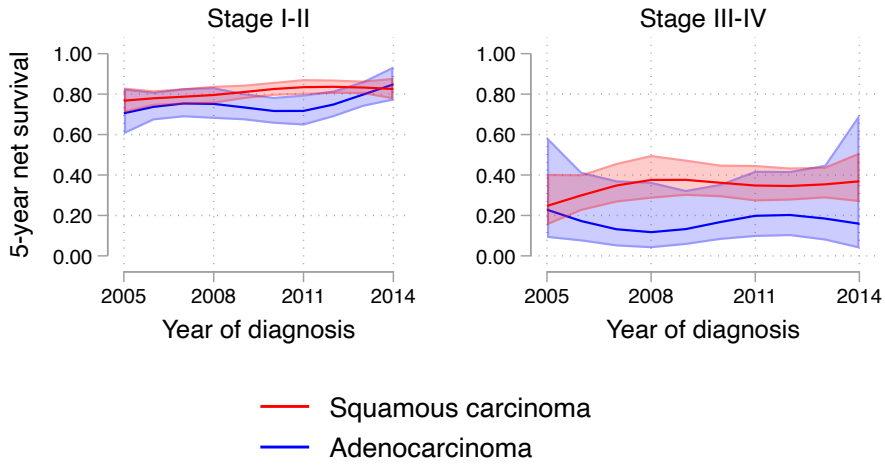

Figure 10. Age-standardized 5-year net survival according to squamous cell carcinoma and adenocarcinoma, stages I-II and III-IV during the time period 2005-2014 with 95% confidence intervals.

Paper II

There were 209 women included in the study with a mean age of 33.7 years (SD 11.1, median 30.0, range 20-68). Three women did not leave a vaginal self-sample, 13 women did not provide a urinary self-sample and two women had missing cervical HPV sample. All available HPV samples could be analyzed. Cytology and cervical histopathological specimens were obtained from 176 women.

The sensitivity in detecting HPV mRNA in the vaginal self-samples and in the urinary self-samples were 83.3% and 48.1% respectively in comparison with HPV mRNA detection by clinician-taken cervical samples (Table 3).

Table 3. Results of HPV mRNA analyses. Sensitivity, specificity, negative predictive value and negative likelihood of vaginal and urine HPV mRNA self-samples compared with clinician-taken cervical HPV mRNA samples.

		Cervical clinician-taken			Self-sampling			
		Pos n	Neg n	Total n	Sensitivity% (95% CI)	Specificity% (95% CI)	NPV% (95% CI)	Negative likelihood% (95% CI)
Vaginal SS	Pos	114	18	132	**83.3** (76.5-89.6)	**73.9** (61.9-83.8)	**69.9** (60.7-77.7)	**0.22** (0.15-0.33)
	Neg	22	51	73				
Urine SS	Pos	63	11	74	**48.1** (39.3-57.0)	**82.8** (71.3-91.1)	**43.8** (39.0-48.8)	**0.63** (0.51-0.76)
	Neg	68	53	121				

HPV: Human papillomavirus.
Vaginal SS: Vaginal self-sampling.
Urine SS: Urine self-sampling.
Pos: Positive.
Neg: Negative.
NPV: Negative predictive value.

The sensitivity of the HPV mRNA assay in detecting HSIL/AIS/cancer was 44.8% for the urinary self-sample, 85.5% for the vaginal self-sample and 100.0% for the clinician-taken cervical sample (Table 4). The corresponding sensitivity of cytology defined as HSIL or worse was 81.7% (95% CI 70.7-89.9) and the specificity was 93.3% (95% CI 86.3-97.3). Two cases of SCC and three cases of AIS were diagnosed in the study. The vaginal self-sample and clinician-taken cervical sample were positive in all of the cases of SCC and AIS, the urinary self-sample was HPV-negative in two cases of AIS and positive in the remaining cases mentioned above.

Table 4. Sensitivity, specificity, negative predictive value and negative likelihood of HPV mRNA testing of vaginal, urine and cervical samples in detecting HSIL/AIS/cancer in histology specimen.

HPV test	HSIL/AIS/Cancer			
	Sensitivity% (95% CI)	Specificity% (95% CI)	NPV% (95% CI)	Negative likelihood% (95% CI)
Vaginal self-sampling	**85.5** (75.0-92.8)	**48.1** (38.2-58.1)	**83.3** (73.2-90.2)	**0.30** (0.16-0.55)
Urine self-sampling	**44.8** (32.6-57.4)	**61.9** (51.4-71.5)	**61.9** (55.4-67.9)	**0.89** (0.68-1.17)
Cervical clinician-taken	**100.0** (94.9-100.0)	**49.0** (39.1-59.0)	**100.0**	**0.00**

HPV: Human papillomavirus.
HSIL: High-grade squamous intraepithelial lesion.
AIS: Adenocarcinoma in situ.
NPV: Negative predictive value.

The correlation between vaginal HPV mRNA and cervical HPV mRNA analyses was $r_s = 0.565$ ($p < 0.01$); urine HPV mRNA and cervical HPV mRNA analyses $r_s = 0.291$ ($p < 0.01$); and vaginal HPV mRNA and urine HPV mRNA analyses $r_s = 0.375$ ($p < 0.01$).

Paper III-V

Table 5 summarizes the results from paper III-V.

Table 5. The results of offering vaginal self-collected samples to long-term screening non-attendees or to women in the upper age screening limit in three different studies (paper III-V) in Sweden.

Paper	Response rate %	HPV+ %	Invalid samples %	Attendance follow-up %	Cervical HPV+ at follow-up %	HSIL+, AIS, Cancer %	HPV+ and normal cytology %
III	43.3	6.2	0.0	100.0	22.2	0.0	20.0
IV	13.2	9.9	0.1	83.5	47.0	1.3 (3 cancer)	29.5
V	18.5	11.3	0.3	85.7	44.8	0.88 (2 cancer)	23.1

HPV: Human papillomavirus.
HSIL: High-grade squamous intraepithelial lesion.
AIS: Adenocarcinoma in situ.

Paper III

The response rate of the HPV self-sample was 43.3% (433/1000, 95% CI 40.2-46.4). HPV mRNA was detected in 6.2% (27/433, 95% CI 4.1-8.9) of the returned samples. Initially 12.7% (55/433) of the samples were invalid by the Aptima HPV assay. After re-analysis or dilution all samples became valid.

All women with detection of HPV mRNA in the self-sample attended the follow-up examination. Six women (22.2%, 95% CI 8.6-42.3) were HPV-positive in the cervical sample at the follow-up. Two women (7.4%, 95% CI 0.9-24.3) at follow-up had cytologically confirmed ASCUS, one tested positive for cervical HPV mRNA and one tested negative for cervical HPV mRNA. No cases of LSIL or worse were diagnosed in the study.

Paper IV

The response rate of the HPV self-sample was 13.2% (797/6,023, 95% CI 12.4-14.1). The mean age of the women who returned their self-sample was 61.2 years (range 33-71). One sample was invalid by the Aptima HPV assay due to insufficient sample material, leaving 796 samples for analysis. HPV mRNA was detected in 9.9% (79/796, 95% CI 7.9-12.2) of the valid samples.

Among women positive for HPV mRNA in the self-sample, 83.5% (66/79, 95% CI 73.5-90.9) attended the follow-up examination. Cervical HPV mRNA was detected in 47.0% (31/66, 95% CI 34.6-59.7) of the women at follow-up. Eight women at the follow-up were diagnosed with high-grade dysplasia at cytology (ASC-H n=3, HSIL n=5). Ten women were diagnosed with high-grade dysplasia or cancer in

histopathological specimens (HSIL n=6, AIS n=1, adenosquamous carcinoma n=1, SCC n=1, ADC n=1,). For these ten women, no cervical smears had been registered in the county of Skåne for 16 years or more. All women with cytological or histopathological diagnosis of high-grade dysplasia or worse were HPV-positive in both the vaginal self-sample and the clinician-taken cervical sample.

There were 13 women positive for HPV mRNA in the self-sample who did not attend the follow-up examination after one reminder letter. A second reminder was given by a telephone call, and if no answer was received, by a second reminder letter. One woman was reached by the telephone call.

Among the 235 non-responding women randomly selected for a telephone interview, three were excluded due to death for an unknown reason. Among the remaining women, 30.6% (71/232, 95% CI 24.7-37.0) were reached by telephone. Eighteen (25.4%) women were excluded due to previous hysterectomy and 27 (38.0%) agreed to participate in the interview. The two most common answers to the question "Why did you not perform the self-sampling?" were that they did not receive a self-sampling kit or they forgot (Table 6).

Table 6. Table showing answers to the question "Why did you not perform the self-sampling?" among women who did not respond to the vaginal hr-HPV self-sampling and agreed participation in a telephone interview.

Reasons for not taking or returning a vaginal hr-HPV self-sample	Women n	Percentage %
Emotional/attitude		
Fear of discomfort	0	0
Feeling healthy	0	0
Phobia/fear of cancer	3	11.1
Ignorance of cervical cancer screening	3	11.1
Insecurity around new test method	1	3.7
Total emotional/attitude reasons	**7**	**25.9**
Practical		
Lack of time	1	3.7
Forgot	5	18.5
Laziness	3	11.1
Too complicated instructions	2	7.4
Total practical reasons	**11**	**40.7**
Physical		
Movement disability restricting self-sampling	0	0
Total physical reasons	**0**	**0**
Needless		
Recent testing elsewhere	0	0
Total needless reasons	**0**	**0**
Other		
Other diseases prioritized	3	11.1
Did not receive a self-sampling kit	6	22.2
Total other reasons	**9**	**33.3**
Total	27	100

Hr-HPV: High-risk human papillomavirus.

Paper V

Results from nine of the offered vaginal self-samples were excluded due to an incorrect invitation. Out of 19,757 correctly invited women, 18.5% (3,646/19,757, 95% CI 17.9-19.0) returned the self-sample. A majority (63.5%) returned the self-sample within one month after the offer (Figure 11). Ten of the returned samples were invalid for Aptima HPV analysis. HPV mRNA was detected in 11.3% (412/3,636, 95% CI 10.3-12.4) of the valid samples.

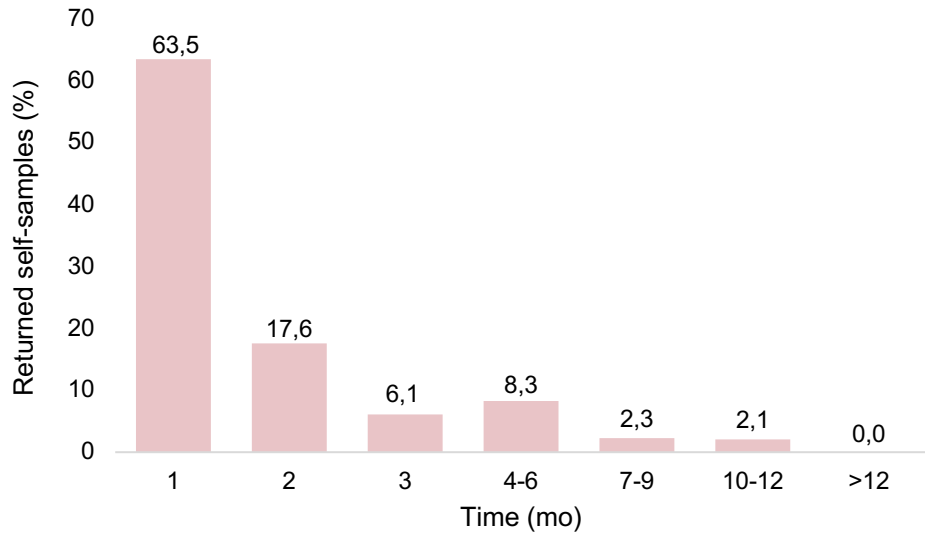

Figure 11. Time in months between offered and returned vaginal hr-HPV mRNA self-collected sample.
Hr-HPV: Human papillomavirus

Of the women positive for HPV mRNA in the self-sample, 85.7% (353/412, 95% CI 81.9-88.9) attended the follow-up. At the follow-up examination, 44.8% (158/353, 95% CI 39.5-50.1) had a positive cervical HPV mRNA sample. There was no statistical difference in cervical HPV positivity rate correlated to follow-up time after a positive HPV self-sample. There were 33 women diagnosed with high-grade dysplasia at cytology. The HPV mRNA assay of self-samples demonstrated a positive predictive value (PPV) of 9.3% for detection of cytological high-grade dysplasia (ASC-H and HSIL). There were 32 women diagnosed with high-grade dysplasia or cancer at histopathological specimens; there were no cervical smears registered for 7-27 years in the registers of the county of Skåne for these women. Two cases of cervical cancer and one case of vaginal cancer were found.

Among women returning the self-sample, the mean age was 52.6 years (range 30-71). A higher response rate of 21.9% was observed in the age group 30-39 years of

age, while the response rate among women ≥40 years old was 18.1% ($p < 0.001$). HPV was more commonly detected in the age group 30-49 years in comparison to women ≥50 years of age (Table 7).

Table 7. Response rate and HPV positivity in vaginal self-collected HPV mRNA analyses stratified by age groups.

Age group (yr)	**Number invited to self-sampling**	**Response rate**		**HPV positive** (n)	**HPV negative** (n)	**HPV prevalence** % (95% CI)
		Number	**%** (95% CI)			
30-39	2,079	455	21.9[a] (20.1-23.7)	66	388	14.5 (11.4-18.1)
40-49	4,592	814	17.7 (16.6-18.9)	106	706	13.1 (10.8-15.6)
50-59	7,937	1,397	17.6 (16.8-18.5)	136	1,257	9.8 (8.3-11.4)
60-71	5,149	980	19.0 (18.0-20.1)	104	873	10.6 (8.8-12.8)
Total	19,757	3,646	18.5 (17.9-19.0)	412[b]	3,224[b]	11.3 (10.3-12.4)

HPV: Human papillomavirus.

[a]P-value 30-39 vs. others <0.001.

[b]After exclusion of 10 returned self-collected samples that could not be analyzed.

Discussion

Incidence of cervical cancer

It is well known that the introduction of a nationwide cervical screening program in Sweden has had a great effect in reducing the cervical cancer incidence. However, the decline in incidence is solely due to a decreased incidence of SCC, while the incidence of ADC has continuously increased between 1960 and 2014 (paper I). A trend of increasing incidence of ADC has also been reported in studies from the USA, and Europe (72, 73). Cervical screening was primarily designed to detect SCC and it is known that cytology is less sensitive in detection of ADC (187). As mentioned earlier, in a nationwide audit in Sweden, it was reported that the screening program was effective in reducing the incidence of ADC (75), though our results in paper I do not support this. In some other countries in Europe, an improved specificity in morphological diagnosis was stated as a potential explanation for the increased incidence of ADC, as the proportion of unspecified malignant tumors declined while diagnosis of ADC increased (73). However, we could not prove such an explanation in Sweden. A possible change in risk factors is unlikely to only affect the incidence of ADC, since SCC and ADC share all co-factors associated with risk of cervical cancer, except smoking which is only reported to increase the risk of SCC (188). The exact reason for the increased incidence of ADC is unknown. However, since ADC is strongly related to hr-HPV infection, the shift to HPV-based cervical screening will hopefully improve the detection of ADC.

Of further interest is that during recent years the decline in SCC incidence has stagnated and a small increase was seen in 2014 (paper I). This has also been noted by other Swedish researches (189). The most significant increase in cervical cancer (30%) was found in women with normal cytology in the last cervical smear before cervical cancer diagnosis (47). Wang *et al*. stated that the increase in cervical cancer incidence was only found among women who had attended screening while no increase was found among non-attendees. Furthermore, there were variations between the 21 counties in Sweden, with no increase in cervical cancer cases in some counties (190). These results suggest changes in screening performance as a large explanation for the increased incidence of cervical cancer (190). Among co-factors associated with risk of cervical cancer there are some that have changed toward an increased risk of cervical cancer. Age at sexual debut decreased and the number of sexual partners increased until 1980; thereafter, no major changes in

these co-factors have been observed (191, 192). Chlamydia trachomatis infection had an incidence peak in 2007, but thereafter decreased (193). The changes in these co-factors are unlikely to contribute to the increased incidence of cervical cancer in later years. Other important co-factors associated with risk of cervical cancer, such as number of full-term pregnancies, age at first birth, use of oral contraceptives and, smoking have changed toward a decreased risk of cervical cancer since the year 1960 (194-198).

Even though Wang *et al*. found it unlikely that changes in risk factors could explain the increased incidence of cervical cancer (190), it is of interest to consider how the prevalence of hr-HPV has changed over time. Finland, Norway, and the Netherlands have also found an increased cervical cancer incidence (42, 44-46). Furthermore, an increased incidence of some other HPV-related cancers (penile, anal, and oropharyngeal cancers) has been observed (41). In a study published in 2002, the hr-HPV prevalence among women 32-38 years old attending regular cervical screening in Sweden was 6.8% (199). In 2017, the corresponding number for women 30-39 years old in the county of Skåne, southern Sweden, was 11.2% (200). However, different HPV assays were used in these studies. From data on HPV prevalence of women aged 15-23 years in Stockholm, Sweden, there is evidence of an increasing prevalence of HPV 39, 51, 52, 56 and 59, but a decreasing prevalence of HPV 16 and 18 in year 2017-2018 in comparison with the years 2008-2010, irrespective of HPV vaccination status (201). In a report with data from 2020 there were signs of stagnation in the increased incidence of cervical cancer in Sweden (202) but data from the coming years is needed to confirm whether this trend is continuing.

Cervical cancer survival

After more than 50 years of organized cervical screening in Sweden, the hypothesis of paper I was that the survival of cervical cancer had improved, which we were able to demonstrate. The age-standardized 5-year net survival of SCC and ADC improved between year 1960 and 2014 (paper I). A similar trend with increasing age-standardized 5-year net survival was seen in several countries in Europe and Asia between year 2000 and 2014 (203). Cervical cancer screening enables detection of cervical cancer at earlier stages which can be considered one important reason for the improved net survival since year 1960 (204). However, in Sweden, it was reported that 64% of all cervical cancer cases and 83% of advanced cases were diagnosed among women who were not adequately screened (75). In a study from Malmö, Southern Sweden, 54% of women diagnosed with cervical cancer had not participated in the screening program according to the guidelines (205). Furthermore, in 2019, 103 cases of a total of 533 cases of cervical cancer in Sweden were diagnosed among women ≥70 years (41) and we noted that more women were

diagnosed in the age group ≥75 years in later time periods (paper I). For these women, improved treatment is the most important factor for determining the cervical cancer survival. As previously mentioned, the greatest improvement in cervical cancer treatment were in 1999 when four articles reported improved survival with the combination of cisplatin and radiation in treatment of patients with locally advanced disease (83-86). Furthermore, the surgical methods and delivery of radiation has improved (206).

Decreasing net survival with increasing age was identified in paper I, which is in concordance with other studies (207-209). One reason to decreasing survival with increasing age is that older women are diagnosed at more advanced stages, which are associated with poorer prognosis (210). In addition, older women might receive less treatment. In a recent Swedish study, it was found that 6% of women in the study population did not receive any primary treatment for their cervical cancer; the median age of these women was 81 years (206). However, treating older patients is complex. Older women more often have comorbid conditions, are more fragile, and the benefit of increased survival must outweigh the potential adverse side effects of cancer treatment. The results from paper I demonstrated an increased or stable 1-, 5-, and 10-year net survival for women 18-64 years old diagnosed with SCC and ADC in 1960 to 2014 but decreased 5- and 10-year net survival for women ≥75 years old. A previous nationwide cohort study in Sweden found it safe to stop the screening of cervical cancer after the age of 60 given normal screening results in the woman's 50s. However, for women under-screened or with abnormal results in their 50s, screening at age 61-65 years reduced the risk of cervical cancer (211). With screening stopping at the age of 64 years today, a woman goes non-screened for 20 years of her life considering that the average life expectancy for women in Sweden is 84 years (212). With HPV-based screening the protection against cervical cancer will be improved (110). Nevertheless, the fact that the long-term survival of our oldest women has decreased during the last 50 years in combination with increasing life expectancy in women indicates that prolonged cervical screening to the age of 75 should be considered.

There are inconsistent results on whether tumor histology is a prognostic factor for cervical cancer survival. Some studies have reported equal survival for SCC and ADC, while others report superior survival for SCC (89, 213-215). The age-standardized 5-year net survival was slightly superior for SCC compared to ADC, but since 2012 no statistically significant difference between SCC and ADC was observed (paper I). The treatment of SCC and ADC has been the same in Sweden during the last decades and our data support the current national treatment guidelines of using similar treatment modalities for the morphologies (204). Better net survival with higher stage at diagnosis was found for both morphologies between 2005 and 2014, which is in line with previous studies (87, 91, 207) and supports the evidence that a stage-shift toward diagnosis at lower stages is important.

HPV self-sampling with mRNA analysis

DNA-based HPV analyses is generally more commonly used than mRNA-based HPV analyses. In paper II we found that urinary- and vaginal self-samples analyzed with Aptima HPV mRNA assay had a sensitivity in detecting HSIL/AIS/cancer of 44.8% and 85.5% respectively in comparison with a sensitivity of 100% of the clinician-taken cervical sample. The sensitivity of the vaginal self-sample was similar to that of routine cytology, but the urinary sample demonstrated a significantly lower sensitivity.

As described in the introduction, there are very few studies investigating the use of an mRNA test for HPV urinary self-sampling. Padhy *et al*. reported a sensitivity of 45.5% in detecting CIN2 or worse in a urinary HPV sample analyzed with Aptima mRNA assay, which cohere with our results (139). However, Arias *et al*. reported that improved HPV positivity and increased agreement between urine and cervical samples analyzed with Aptima mRNA assay can be achieved by direct stabilization of first-void urine in transport medium followed by the addition of Proteinase K (216). In our study, initial stream urine was used and direct transfer into a transport medium was performed, but there was no addition of Proteinase K. Senkomango *et al*. investigated the collection procedure of a urinary sample and found that hr-HPV prevalence was similar in first-void, initial stream, and mid-stream urine for unfractionated and pellet fractions (135). Several studies have demonstrated comparable results for urinary and cervical DNA-based HPV samples (126, 133-135). The lower sensitivity found for the urinary sample in paper II could be explained by the choice of preservative solution, storage condition or testing volume, and/or the fact that an mRNA assay was used.

In paper II the sensitivity to detect HSIL/AIS/cancer was higher in clinician HPV mRNA sampling compared to vaginal HPV mRNA self-sampling. However, since paper II was conducted, the Aptima mRNA analysis has been improved by adding a pre-heating step where self-samples are preheated at 90 °C for one hour before analysis (217). Samples included in paper II which were mRNA negative in the vaginal self-sample and mRNA-positive in the cervical sample were re-analyzed after the performance of the pre-heating step. The results of this re-analysis showed that 55% of previously negative vaginal self-samples became positive and when updating our series with these results the sensitivity of the vaginal mRNA self-sample to detect CIN2+ was 95.3% (217). Furthermore, the study found an increased proportion of valid results after the pre-heating step (217). Two previous studies have found a sensitivity of 86.7% and 100% respectively to detect CIN2+ in self-samples analyzed with the Aptima mRNA assay (218, 219). The corresponding pooled sensitivity for self-samples analyzed with polymerase chain reactions was 96% in Arbyn's meta-analysis (124). The sensitivity of the vaginal self-sample in detecting HSIL/AIS/cancer in paper II was similar to routine cytology, which supports the use of vaginal self-samples analyzed by Aptima HPV mRNA assay in

a screening setting for non-attendees. However, because of the increased sensitivity due to the pre-heating step in combination with results from another study in which we demonstrated similar detection of high-grade dysplasia of a vaginal self-sample and a cervical sample analyzed for Aptima mRNA with the pre-heating step, the vaginal HPV mRNA self-sample can be considered safe to use for all women in a screening setting (130, 217).

Reaching screening non-attendees

The county of Skåne is one out of six other counties in Sweden offering self-collected samples to long-term screening non-attendees (123). Several studies have demonstrated a higher response rate to self-samples compared to routine screening among screening non-attendees (97, 143, 151). In paper IV and V, the response rates to opt-out self-sampling tests were 13.2% and 18.5% respectively. Screening non-participants can be invited to self-sampling through several strategies, the most common being opt-out (mail-to-all), opt-in or door-to-door. In a meta-analysis by Arbyn *et al*. it was reported that delivery of a self-sample to a woman's home by a health worker (door-to-door) resulted in the highest participation rate, with a pooled response rate of 94.2%; however, this is very time-consuming for the health personnel. The most used invitation strategy was opt-out, with a pooled response rate of 19.2%. Among women who had to order the self-sample themselves (opt-in), the pooled participation rate was lower (7.8%) in this meta-analysis (124). However, some studies have reported good response rates to opt-in of 16-39% (140, 141, 220). A Danish study offering self-samples to non-attendees through opt-in presented four different ways to order a self-sample: ordering the self-sample through a web page, a phone call, or an e-mail, or returning a reply form through regular mail. In total, 32% ordered the self-sample and 20% returned it (185), which is in accordance with our results in paper V. Regular mail followed by the web page were the two most common ways to opt into the study (185). In our studies, the response rate significantly increased between 2017 (when study IV was conducted) and 2018 (when study V was conducted) which is positive (13.2% vs 18.5%, $p<0.001$). The exact reasons for this are not known. However, it could be speculated that the increased response rate was due to a rise of knowledge and acceptance of self-sampling in the society.

Comparing our results with other Swedish studies, several studies have reported higher responses to HPV self-sampling. Three studies reported responses of 32%, 34% and 39% to HPV self-sampling among women with no cervical sample for $\geq$6 years, two studies used an opt-in and one an opt-out invitation strategy (140, 143, 220). Broberg *et al*. reported that 16% of non-attendees returned an HPV self-sample in an opt-in strategy and 8.5% chose to visit a midwife clinic for a cervical smear, rendering a total response rate of 24.5% in the non-attendees group (141).

Lilliecreutz *et al*. found a response rate to self-sampling among women with no cervical smear for ≥6-8 years of 26.2%, and in addition 8.6% took a cervical smear at a midwife clinic (151). A common factor for all of the other studies is the use of reminder letters. A previous study in the county of Skåne found a rather low response rate to HPV self-samples among non-attendees of 4.9%; however, after a reminder letter, including a new self-sample, the response rate increased to a total of 14.7% (97). Lilliecreutz *et al*. also reported a rise in response rate from 16.6% to 26.2% after a reminder letter (151). In paper V it was demonstrated that 81.1% of participating women had returned their self-sample within two months after the offer. A reminder letter sent two months after the initial offer could have been an approach to increase the response rate to self-sampling and should be recommended.

Another possible way to increase the screening attendance among non-participants is to use telephone calls, which is recommended in the national screening program in Sweden (94). One previous study from Sweden found a participation rate to cervical screening of 18.0% after non-attendees received a phone call with an offer of an appointment for cervical sampling at a midwife clinic (221). Lilliecreutz *et al*. found a participation rate to cervical screening of 19.7% among women receiving a phone call with an offer to receive a self-sample or an appointment for a Pap smear, another 8.3% provided a pap smear after the invitation letter without answering the phone (151). Telephone calls reach around one fifth of screening non-attendees; however, it can be difficult to reach the women due to missing/wrong telephone numbers or the women not answering their telephone. In the studies mentioned above, 81.6% with a maximum of ten attempts and 62.2% with a maximum of three attempts respectively of the called women were reached (151, 221). This shows that the process is rather time-consuming, and the telephone calls were not found to be more effective than a self-sampling offer (141, 151). In paper IV, telephone contact was attempted with women with a positive HPV self-sample not attending the follow-up examination, but only one out of 13 women were reached.

Reasons for not returning a self-sample were reported in previous studies to be opportunistic screening outside the screening program, uncertainty about correct performance, preference of regular screening, the opinion that screening was unnecessary, pregnancy or previous hysterectomy (142, 146-148). In study VI, only 30.6% were reached when called a total of three times and 11.6% participated in the telephone interview on why they did not return their self-sample. The results need to be interpreted with caution because of the low participation rate. The reason for the low number of reached women could not be because of the time of the day the calls were made since every call was made at different times and a previous study found that calls resulting in contact were evenly distributed throughout the day (221). The two most common reasons for not returning the self-sample were “did not receive a self-sampling kit” and “forgot”, followed by four equally common answers “phobia/fear of cancer”, “ignorance of cervical cancer screening”, “laziness” and “other diseases prioritized”. Forgetting to take a cervical screening

sample was reported as a common reason for not attending regular screening as well (97). A reminder letter could have served a good purpose for these women. It is noteworthy that more than a fifth of the women reported that they did not receive the self-sampling kit. An invitation letter before sending out the self-sampling kit or an opt-in strategy would have made the woman aware if the self-sampling kit was not delivered. Even though Arbyn *et al*. reported a lower participation rate with an opt-in strategy (124), several Swedish studies have shown a high participation rate for opt-in why this could be an option (140, 141, 220). No woman reported "feeling healthy" as the reasons for not returning her self-sample which was the second most common reason for not attending regular screening in a previous study (97). Furthermore, only one woman answered "lack of time" as the reason for not returning her self-sample. This reason was the third most common one for not attending regular screening in a previous study and proves that self-samples overcome this issue (97).

HPV prevalence and cervical dysplasia among non-attendees

The cervical HPV prevalence in the screening population (30-70 years old) in the county of Skåne was 7.0% in 2017 (200). This is lower compared to the vaginal hr-HPV prevalence among the responders of paper IV and V, which was 9.9% and 11.3% respectively. In other Swedish studies the vaginal hr-HPV prevalence among non-attendees varied between 6 and 13% (97, 140-143, 151) and one study found a higher hr-HPV prevalence of 26% (220). The pooled hr-HPV prevalence in vaginal self-samples among never- or under-screened women in 22 studies included in the meta-analysis by Arbyn *et al*. was 11.1%, which is in accordance with our results (124). One possible explanation for the slightly higher hr-HPV prevalence among non-attendees in the county of Skåne in comparison with the screening population might be that screening non-attendees are reported to more likely be single than cohabiting or married (222, 223). It can be expected that single women have more sexual contacts than cohabiting or married women, and the number of sexual contacts increases the risk of acquiring an HPV infection (57).

For HPV self-sampling to be beneficial, a high compliance to follow-up is fundamental. The compliance to follow-up among HPV-positive women in the self-sample was 83.5% in paper IV and 85.7% in paper V. This can be compared to an average participation rate to follow-up of 80.6% in the meta-analysis by Arbyn *et al.* (124). Screening non-participating women have expressed a reluctance to have a gynecological examination (97). One way to reduce the number of women needing a gynecological examination is to use repeated self-sampling as follow-up strategy. Gustavsson *et al*. applied the use of repeated self-sampling in a study of a group of

women 30-49 years invited to routine screening with HPV self-sampling. Of the women positive for HPV in the first self-sample, 90% returned a second self-sample and 6.7% chose to attend a health care clinic for follow-up, rendering a total participation rate at follow-up of 96.7%. At follow-up, 71% of women were continuously HPV-positive and these were invited to colposcopy (224). This method resulted in a very high participation rate at follow-up and reduced the reference rate to colposcopy by at least 30%. Furthermore, it had a higher detection rate of CIN2+ in comparison with cytology and was cost-effective for the health care (186, 224).

In study IV and V, 53% and 55% of women with positive self-samples had turned HPV mRNA-negative in the cervical HPV sample at follow-up. The corresponding numbers in previous studies using HPV DNA analyses were 27-41% (140, 149, 225). It is not fully understood if the conversion to a negative HPV test is due to clearance of the HPV infection, persistent infection which does not reach the threshold for detection, different HPV flora of the vagina and cervix, or the analytic method. In a recent study in the county of Skåne using the Aptima assay, it was found that 63% of women who were initially HPV-positive in a vaginal self-sample had turned HPV negative in a cervical sample. Among women initially HPV positive in a cervical sample, only 28% had turned HPV negative in a renewed cervical sample (130). Thus, a higher proportion of women positive in the self-sample tested negative in the cervical sample, which might indicate that the mRNA test to a certain extent detects some infections only localized to the vagina. However, of importance is that all women diagnosed with histologically LSIL, HSIL or cancer tested HPV mRNA-positive in both the self-sample and the cervical sample in our studies. Of further interest is that 29.5% (paper VI) and 23.1% (paper V) of women with benign cytology tested positive for HPV mRNA in the cervical sample. The corresponding number among women aged 40-42 years in the regular screening program in the county of Skåne was 4% (200), which is significantly lower and indicates non-attendees to be at higher risk of development of cervical dysplasia.

We found that the prevalence of high-grade dysplasia or cancer in paper IV and V was similar or somewhat lower in comparison with results from the national cervical screening program in Sweden (226). However, the prevalence of solely cervical cancer was found to be almost seven times higher in comparison with women in organized screening in study IV (0.4% in study IV, 0.06% in organized screening in Sweden 2016 and 2017), but similar to organized screening in study V (0.06% in study V) (226). Other studies of screening non-attendees have found a cervical cancer prevalence of 0-1.0% among women with no cervical sample for $\geq$5-9 years (97, 142, 150, 227). The reason to a relatively variating prevalence of cervical cancer among long-term non-attendees is probably because of varying times since the last screening sample, since the risk of cervical cancer increases with greater time between cervical samples. However, it is important to remember that the total

number of cervical cancer cases in our studies is low, and each additional or non additional cancer case has a large influence on the prevalence. Nevertheless, it is well known that screening non-attendees have an increased risk for cervical cancer (75) and the fact that we found an almost seven times higher cervical cancer prevalence in study IV strengthens this fact.

Cervical screening among older women

In terms of the potential need to raise the upper age limit of screening, more knowledge of women older than those included in the current screening program is needed. The screening program has developed from an upper age limit of 49 years old at the end of the 1960s (228), to the current recommendations implemented in 2015 with screening of women until the age of 64 years (and yearly recalls up to the age of 70 years if no sample was registered at the age of 64 years)(94). However, in 2020, the screening coverage of women aged 61-70 years was only 55.2% (96). Vaginal self-sampling is reported to be easy to use and preferred over clinician sampling among older women (229). In paper III we found that 43.3% of women aged 69-70 years accepted and returned a free-of-charge offered vaginal HPV self-sample. In a previous Swedish study, 59.5% responded to a vaginal HPV self-sample among women in four age groups 60-75 years old (107). Another study found a response rate of 39.4% by opt-in self-sampling among women aged 50-65 years with no cervical sample for ≥6 years (144). These response rates are higher in comparison with studies offering self-samples to screening non-attending younger women (124). A higher response rate among women 69-70 years old in our study could be because some of these women might not have been screening non-attendees. Since an inclusion-criterion of the study was that women should have no registered cervical sample for ≥5 years, some women could have taken a cervical sample at 64 years old, which is the last sample taken in the organized screening program. Women that have attended screening regularly throughout their life will be more aware of when they are above screening age and aware of the benefits of screening, which can influence the attendance toward a higher rate. For comparison, in paper V the response rate to self-sampling of women aged 60-71 years, was only 19.0%. In paper V the women had no registered cervical sample for ≥7 years which made most women non-attendees, which was reflected in a lower participation rate. Furthermore, study III was conducted in the municipality of Lund in which a greater proportion of the population have a high level of education in comparison with the county of Skåne where study V was conducted (230). A high level of education among older women has been shown to correlate with increased participation to self-sampling (231).

The HPV prevalence among women aged 69-70 years old was 6.2% in paper III. Previous studies have reported an HPV prevalence between 3.8 and 7.4% among

women 50-90 years of age (104, 107, 144, 232-236). All women attended the follow-up examination; however, only 22.2% tested positive for HPV mRNA in the cervical sample (paper III). This is a surprisingly low proportion. Other studies have found a loss of detection of HPV DNA or mRNA of 37-48% for postmenopausal women after 4-12 months which is substantially lower than the 78% loss of detection in our study, although these studies used repeated cervical samples and not an initial self-sample as used in our study (104, 237, 238). However, in studies with repeated self-sampling the loss of detection of HPV DNA was 43.6% in one study among women aged 60-75 years and 39% in another study of women ≥50 years of age (107, 236). Stanczuk *et al*. found the hr-HPV DNA positivity to be 38% higher in vaginal self-samples in comparison with cervical samples among women ≥50 years possibly because of menopausal changes of the cervix (137). But Gravitt *et al*. found excellent agreement between a vaginal HPV DNA self-sample and a clinician HPV DNA sample irrespectively of age and menopausal status (239).

There were only two cases of ASCUS in cytology and no cases of high-grade dysplasia or cancer detected among the women participating in study III. However, a limitation was that a histopathological specimen was only collected for one woman; for all other women, only cytology was performed at follow-up. Several studies have proven a low sensitivity of cytology among postmenopausal women (103-107). However, this is still the triage-method recommended after a positive HPV sample among postmenopausal women in the organized cervical screening (94). Interestingly, the prevalence of histological cervical dysplasia was found to be 45% among women 70 years of age positive for cervical HPV mRNA in a recent study (233). Another study found a prevalence of histological cervical dysplasia in 81% (CIN 1 n=18, CIN 2 n=4) of women aged 60-89 years old whom were HPV-DNA-positive in two consecutive samples; 86% of these women presented with normal cytology (104). This indicates that triage methods for HPV-positive older women need to be changed and adapted to current knowledge.

Even though no high-grade lesions or cancer were found in our study, an HPV prevalence of 6.2% (paper III) indicates a risk of development to dysplasia and it is important to continue the follow-up of older HPV-positive women. Asciutto *et al*. found that 29.7% of women aged 60-65 years positive for hr-HPV mRNA and with normal cytology at baseline, developed histologically high-grade dysplasia during a surveillance period of 49 months. The corresponding proportion for hr-HPV DNA positive women was 3.6%. None of the HPV mRNA-negative women developed high-grade dysplasia, demonstrating the high specificity of the mRNA analysis (237).

Strengths and limitations

Paper I

Paper I is a nationwide population-based register study with longitudinal data on cervical cancer in Sweden for over 50 years, which is a major strength of the study. The completeness of the SCR is high (>95%) and >98% of cases have morphologic verification of the diagnosis (177, 178). Incidence rates age-standardized to the World Standard population were used, which facilitates international comparison and minimizes confounding from changing population patterns. The use of net survival as an estimation for survival enables comparison between different populations or within the same population over time since it is independent of mortality, this increases the internal and external validity of the study. Net survival has not commonly been used in clinical research so far and the definition may be perceived as complicated. The term relative survival has been more commonly used, but life table estimates of relative survival are biased. In paper I we estimate net survival in a relative survival framework using a model-based approach, which does not suffer from this bias, which is a strength. Furthermore, among leading groups working in the field, net survival is the currently accepted term. To our knowledge, this study is the first to analyze time trends of net survival in different subtypes and age groups of cervical cancer.

A weakness of the study is the absence of central pathology. In addition, time trends for early time periods of stage could not be calculated because stage was not included in the SCR until 2004. An important bias to consider is the improvements in histopathological diagnosis since 1960. Information about treatment is not available in the SCR; therefore, our results could not be directly correlated to different treatment regimens used in the different time periods.

Paper II

To be able to accurate diagnose a patient is of great importance since all therapeutic interventions are based on the presumptive diagnosis. In the case of screening, it is also of great importance to be able to rule out disease so as to avoid overtreatment and unnecessary anxiety for the patient. Study II was performed as a diagnostic accuracy cross-sectional study. The use of a cross-sectional study has the benefit of being relatively easy, cheap, and fast to perform. In our study, the women served as their own control, which minimized bias. All samples were collected during the same day for each woman. The HPV samples were analyzed with the same assay, the Aptima mRNA assay, in the same laboratory in Lund, Sweden. All HPV samples could be analyzed. Histopathological specimens obtained from a cervical biopsy or the LEEP procedure were used as reference standard, which is a strength of the study since histopathological specimens are considered as reliable data.

An important limitation of the study is that it was performed in a referral population with a higher prevalence of hr-HPV infection and cervical dysplasia which limits the external validity of the study. Positive and negative predictive values are known to directly depend on disease prevalence, which limits the comparison of our results in general screening settings. Further studies in a screening population are needed, and in 2019 we conducted a randomized controlled trial in a screening population to confirm the results (130). Another weakness is that the women performed the self-sampling at the clinic and not at home, which would have been the most relevant setting in terms of the possibility to use self-samples in a screening program. Both oral and written instructions on how to perform the self-sampling were given to the patients, which might have increased the likelihood of the patient correctly performing the sampling. The process of sending the self-samples through the mail, which would have been the situation if the woman performed the self-sampling at home, might also affect the sample. However, the Aptima mRNA sample is reported to be able to withstand temperatures between 2-30 °C and a sample can be stored for up to two months.

Paper III-V

The great strength of paper IV and V was that the studies were performed in a setting of non-attendees of a current population-based cervical screening program, which improves the generalizability of the study. Paper III had a smaller study population, and the generalizability of that study might be limited; however, the response rate was high in paper III, which is a strength. When introducing a new test method, it is important that this method is accurate and provides a low number of invalid test results. The number of invalid self-samples in paper III-V was 0-0.3% which is considered a strength. However, in paper III, initially 12.5% of the self-samples were invalid, but became valid after dilution. In paper IV, as many as 25% of samples in one batch were initially invalid and became valid after dilution. Dilution of samples removes potential inhibitory substances. However, a disadvantage of dilution is that the process can make the HPV diluted so that it can no longer be detected due to too few HPV mRNA copies in the sample. With the introduction of a pre-heating step prior to analysis of self-samples, the high proportion of initially invalid samples is expected to decrease (217). Loss-to-follow-up is an important weakness of a cohort study. In our studies the loss-to-follow-up was considered low as all women attended follow-up in study III and around 85% attended follow-up in studies IV and V.

In the registers in the county of Skåne we can only access information about previous cervical samples taken in the region and we do not have access to screening history in other parts of Sweden. If a woman has taken a cervical sample in another county in Sweden she might be wrongly registered as a screening non-attendee in the county of Skåne which is a limitation of the studies. This could have been avoided by adding an inclusion criterion that each woman should have been

registered at an address in the county of Skåne for a certain time. However, most other counties in Sweden share a mutual system for cervical screening and follow-up (Cytburken) and it would be ideal if all counties joined this system. A weakness of all three studies is that women with a previous hysterectomy, which is a criterion of exclusion of cervical cancer (94), were not excluded from the studies. Approximately 5-6% of women aged 40-60 years in Sweden have had a total hysterectomy (240). In the national cervical screening program, women that have undergone a total hysterectomy are supposed to be excluded from invitation by being added to a specific "block list". Yet, in the follow-up of patients in the studies it was noticed that several had undergone a total hysterectomy. This can reduce the participation rate since most of these women are aware that they do not need cervical screening. To avoid incorrect invitations to cervical screening, the routine of adding total hysterectomized women to the "block list" should be reviewed. We did not collect lifetime information about the study participants. For example, sexual history, history of sexually transmitted infections, education, income, partnership, and part of the labor force or not are known factors that can affect the attitude toward screening (222, 241). However, even though these factors might improve the understanding of why we found certain results, it is relatively well studied before and was not part of the aim of our studies. No reminders were sent out in the studies, which has previously been discussed as a weakness. We did not investigate whether women provided a cervical sample at a midwife clinic instead of returning the self-sample after the self-sampling invitation. The aim of the studies was to explore the response rate to self-sampling; however, if a screening non-attending woman chooses to attend a midwife clinic for a cervical sample instead, this must be seen as a successful outcome. In previous studies, around 8% of women invited to self-sampling chose to attend a midwife clinic instead (141, 151). As previously mentioned, a limitation of study III was that histopathological specimens were not collected as part of the routine follow-up for HPV-positive women. With the low proven sensitivity of cytology among postmenopausal women, it is possible that the proportion of precancerous lesions was underestimated in our study. However, the follow-up protocol was designed according to current guidelines of the national cervical screening program.

Conclusions

- After the introduction of cervical cancer screening, the age-standardized incidence of SCC has decreased until the year 2000 and the incidence of ADC has increased between 1960 and 2014 (paper I).
- Short- and long-term net survival improved for all women 18-64 years of age diagnosed with cervical cancer between 1960-2014, except for long-term net survival among women ≥75 years of age, suggesting prolonged HPV screening up to 75 years of age (paper I).
- Age and stage at diagnosis were important prognostic factors in determining net survival for cervical cancer (paper I).
- There was no clinically significant difference in net survival between SCC and ADC after 2012 (paper I)
- A vaginal HPV self-sample analyzed with Aptima mRNA analysis in 2015-16 had a similar sensitivity in detecting HSIL/AIS/cancer as routine cytology and may be used as a complement in cervical screening to reach screening non-attendees (paper II).
- A urinary HPV sample analyzed with Aptima mRNA analysis demonstrated a sensitivity that was considered too low to use it as a screening test (paper II).
- Vaginal HPV self-samples were accepted among women 69-70 years of age. The prevalence of HPV mRNA was 6.2%, but no high-grade dysplasia was found in cytology (paper III).
- The response rate to vaginal HPV self-samples among long-term non-attendees increased between 2017 and 2018. In 2018, almost one fifth of women returned their self-sample, suggesting self-samples to be a promising method to increase attendance to screening among non-attending women (paper IV and V).
- The hr-HPV prevalence in self-samples was higher among long-term non-attendees compared to cervical sampling among women in organized screening, but the results were varied regarding the prevalence of cervical cancer in non-attending women in the two papers (paper IV and V).

Future perspectives

In the near future, self-sampling tests will be an opportunity for all women scheduled for cervical screening. In a randomized controlled trial in the county of Skåne in the south of Sweden, we found that vaginal self-sampling with Aptima mRNA analysis detected a similar proportion of high-grade dysplasia as regular screening among women 30-64 years old (130). These results have led to the decision to replace all cervical cancer screening in women 23-70 years of age with HPV self-sampling in the county of Skåne from the autumn of 2021. One other county in Sweden has made the same conversion to screening with self-sampling and it is expected that this will be implemented in more regions. Self-sampling tests have practical benefits for women and economic benefits for society in that women do not have to visit a clinic during office hours. With self-samples, midwives will have fewer cervical samples to take and can use the extra time for other health care. Furthermore, cost calculations suggest that self-samples are cheaper for health care compared to clinician-taken cervical samples (242). In the randomized controlled trial the attendance to screening was significantly higher in the group invited to cervical sampling at a midwife clinic compared to the self-sampling group (130). However, the attitude to a new method in the frames of a research study might make some women insecure, which could have affected the participation rate in the self-sampling group. As HPV self-sampling is now implemented as a primary screening method this will hopefully improve the attitude to self-sampling, especially since previous studies have revealed that HPV self-sampling is preferred over clinician sampling by most women (132).

Furthermore, with the introduction of HPV testing for all ages there is a hope that the incidence of ADC, of which cytology has a low sensitivity to detect (74), will decrease. Some studies have reported an increased incidence of ADC, especially among young women (72, 243). This might be because HPV sampling has not been used for primary screening among women <30 years old due to a higher HPV prevalence representing mostly transient HPV infections among younger women. However, it is now 15 years since the HPV vaccine was introduced and nine years since it was implemented in the national vaccination program for all girls in grades 5 and 6 in Sweden. In 2016, 35.7-62.9% of women who are 23-29 years old today had taken at least one dose of HPV vaccine; the only age-category where fewer than 50% of the women had taken the HPV vaccine was women who are 29 years old today (244). Among women who received the HPV vaccine as part of the

vaccination program, the vaccine-coverage is >80% (244). Hence, the HPV prevalence will be reduced because of a high vaccine-coverage which, in combination with HPV testing being superior to cytology (110), motivates the usage of HPV screening also among younger women.

Another aspect of HPV self-samples is the potential to use them as a follow-up method after treatment of high-grade dysplasia. A recent Swedish study revealed similar sensitivity of an HPV DNA self-sample as of an HPV DNA cervical sample for predicting post-treatment outcome for women with squamous histopathology. However, among patients with glandular histopathology the self-sample did not demonstrate sufficient sensitivity (245). Furthermore, the possibility to use self-samples as a test of cure has been proposed (128).

HPV tests have a very good sensitivity in detecting high-grade cervical lesions or cancer; however, the specificity is lower (112), and triage of positive HPV tests is necessary to avoid over-referral and overtreatment. A draw-back of vaginal HPV self-samples is the inability to perform reflex testing for cytological analysis. It would be ideal to perform the triage test on the same sample as the self-sample as to minimize loss to follow-up. Molecular reflex tests based on hypermethylation is a promising method. One advantage of the molecular biomarkers is the removal of the human factor since they are machine-read in contrast to the subjective method of cytology. In a recent meta-analysis of 43 studies including different gene markers, it was found that DNA methylation increased with increasing grade of CIN (246). Furthermore, the DNA methylation assay demonstrated higher specificity compared to cytology (ASCUS+) and higher sensitivity compared to HPV 16/18 genotyping (246). We are currently working on a study analyzing the use of DNA methylation in the human genes FAM19A4 and miR124-2 as a potential triage method in cervical screening. In a large European study, DNA methylation of these genes has shown promising results, proving triage with DNA methylation to be equal or better than cytology triage (247).

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Cervical cancer

Cervical cancer is preventable by vaccination and screening. Sweden introduced a nationwide cervical screening program in late 1960s. This thesis analyzes the incidence of cervical cancer in Sweden 1960 to 2014 which has decreased, and net survival which has improved. Not attending to the screening is a large risk factor for cervical cancer. This thesis shows that the use of vaginal HPV self-samples analyzed with an HPV mRNA assay can be used as a complement to screening to reach non-attendees. Furthermore, the use of self-samples has in three papers in this thesis shown to improve attendance to screening and discovery of treatable precancerous lesions and cancer.

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