Cervical cancer prevention, evaluation of HPV analyses in the screening programme

Antgren Hellsten, Caroline

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CAROLINE HELLSTEN
DEPARTMENT OF CLINICAL SCIENCES, LUND | FACULTY OF MEDICINE | LUND UNIVERSITY

CAROLINE HELLSTEN is a resident in obstetrics and gynaecology at Skåne University Hospital, Sweden. The overall aim of this thesis is the prevention of cervical cancer through the evaluation of the screening programme, with particular emphasis on the HPV self-sampling device, while incorporating the perspectives of women participating in the screening programme.

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Caroline Hellsten

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Abstract

Cervical cancer continues to represent a significant global public health burden, despite progress in both screening initiatives and vaccination programmes. High-risk human papillomavirus (hrHPV) testing offers greater sensitivity than cytology for detecting cervical precancerous lesions but with lower specificity. Recent advancements have introduced vaginal self-sampling as an alternative method suitable for HPV testing. Enhancing programme design and understanding women's experiences are critical for improving clinical practice and advancing efforts toward cervical cancer elimination. The overarching aim of this thesis is the prevention of cervical cancer through the evaluation of the screening programme, with particular emphasis on the HPV self-sampling device.

Paper I presents the results of a randomised controlled trial. Vaginal HPV self-sampling kits were mailed to screening-eligible women, and follow-up appointments were scheduled for HPV-positive women. The control group consisted of women directly invited for midwife-collected cervical HPV screening. The participation rate in the self-sampling arm was 33.5% and HPV prevalence 17.1%. In the control group 47.5% of the invited women had samples taken by midwives and HPV prevalence was 4.5%. The self-sampling approach detected a comparable proportion of high-grade dysplasia to that identified through conventional screening methods (0.48% versus 0.47%). Thus, our study indicates that self-sampling could replace primary HPV screening of cervical samples. **Paper II** reports a quality assurance audit. The data were collected from the National Cervical Cancer Prevention Registry, Region Skåne Labmedicin database and the Melior Journal system in 2017-2020. We identified 247 women diagnosed with invasive cervical cancer. Non-participation in the screening programme was the main factor associated with invasive cervical cancer (143, 57.9%), followed by women being above screening age (44, 17.8%). Consistent participation in cervical cancer screening programmes remains the most effective strategy for prevention. **Paper III** has a qualitative study design with content analysis using an inductive approach. A questionnaire with nine open-ended questions and nine background questions was used to collect narratives from screening eligible women. The HPV self-test reduced practical and emotional barriers to attending the cervical cancer screening programme, but test results may create anxiety. **Paper IV** evaluates the implementation and outcomes of the self-sampling programme in a real-world setting. Data were collected using the Laboratory Information Management System (LIMS) and the Melior Journal system. The return rate for vaginal self-sampling kits was 37%, with an HPV prevalence of 19%. Of those requiring follow-up, 81% completed their appointments, and 39% were still HPV-positive. The proportion of biopsy-verified high-grade cervical dysplasia or cancer was 0.5% (1,012/204,763) of all eligible self-samples. The direct distribution of self-sampling kits has proved effective in facilitating HPV detection and enabling early intervention, highlighting its potential to contribute to cervical cancer prevention even in populations with initially low screening compliance.

Key words: Cervical cancer, human papillomavirus, vaginal self-sampling, organised cervical screening, acceptability, experience, questionnaire

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Caroline Hellsten

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Faculty of Medicine

Department of Obstetrics and Gynaecology

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To my family, for your love and endless support

First comes the heart. The heart must be associated with the sentiment of humanity, and that's what liberty must correspond to; a free mind. We are free beings, we must have a great heart and a great humanity."
– Taiji Kase, the karate master in Shotokan Ryu and Karate Do (1929-2004)

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I. **Hellsten C,** Ernstson A, Bodelsson G, Forslund O, Borgfeldt C. Equal prevalence of severe cervical dysplasia by HPV-sampling and by midwife-collected samples for primary HPV-screening: a randomised controlled trial. *Eur J Cancer Prev. 2021;30(4):334-40.*

II. **Hellsten C,** Holmberg A, Astrom J, Forslund O, Borgfeldt C. Cervical cancer in Region Skåne, Sweden 2017-2020 after the implementation of primary HPV screening: a quality assurance audit. *Acta obstetricia et gynecologica Scandinavica. 2024;103(1):129-37.*

III. **Hellsten C,** Borgfeldt C, Magnusson L. Women's reasoning and experience in the cervical cancer screening programme when offered a self-sampling HPV-test: a qualitative content analysis. *Submitted.*

IV. **Hellsten C**, Forslund O, Borgfeldt C. Real-world evaluation of a population-based vaginal self-sampling program for cervical cancer prevention in Region Skåne, Sweden. *Accepted.*

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

Sundqvist A, **Hellsten C,** Strander B, Lindh M, Borgfeldt C. Self-collected vaginal HPV samples for long-term screening non-attendees in the region of Västra Götaland. *Acta Obstetricia et Gynecologica Scandinavica. 2025;104(6):1181-9.*

Abstract

Background:
Cervical cancer remains a significant global public health challenge, despite advances in both screening initiatives and vaccination programmes. In high-income countries, most cervical cancer cases are attributable to irregular participation in screening. Persistent infection with high-risk human papillomavirus (hrHPV) is the principal cause of nearly all cervical cancer cases. Compared with cytology, hrHPV testing offers higher sensitivity but lower specificity for detecting cervical precancerous lesions and can also be performed on self-collected samples. Recent innovations have introduced vaginal self-sampling as an alternative screening method, demonstrating diagnostic accuracy comparable to clinician-collected samples. HPV self-sampling provides several advantages, including the possibility of extending screening intervals, reducing barriers to participation, and lowering healthcare costs. Targeted improvements to screening programmes are essential for the successful eradication of cervical cancer in the future. Furthermore, understanding women's experiences of cervical cancer screening is critical, with important implications for clinical practice.

Objective:
The overarching objective of this thesis is the prevention of cervical cancer through the evaluation of the screening programme, with particular emphasis on the HPV self-sampling device.

Paper I: To investigate compliance, the prevalence of HPV, and the prevalence of severe dysplasia in participants using vaginal self-sampling, compared with participants undergoing cervical sampling performed by midwives (control arm).

Paper II: Characterize the screening history of women diagnosed with cervical cancer to evaluate the performance of the screening programme, as well as to assess the cancer treatments given and shortcomings in the follow-up of women with cervical dysplasia.

Paper III: Explore women's reasoning and experience when offered a self-sampling HPV test in the screening programme for cervical cancer.

Paper IV: Evaluate the implementation and outcomes of the HPV self-sampling programme in a real-world setting.

Methods:
Paper I presents the results of a randomised controlled trial. Vaginal HPV self-sampling kits were sent by regular mail to 14,765 screening-eligible women. HPV-positive women were invited for a follow-up examination by their midwife in which they provided a cervical sample for cytological and HPV co-testing. The control group consisted of 14,839 women directly invited for midwife-collected cervical HPV screening.

Paper II reports a quality assurance audit. The data were collected from the National Cervical Cancer Prevention Registry, Region Skåne Labmedicin database and the Melior Journal system in 2017-2020.

Paper III has a qualitative study design with content analysis using an inductive approach. A questionnaire with nine open-ended questions and nine background questions were sent to women due for screening aged 23-64.

Paper IV evaluates the implementation and outcomes of the HPV self-sampling programme in a real-world setting. Data were collected using the Laboratory Information Management System (LIMS) and the Melior Journal system.

Results and conclusions:
Paper I: The participation rate in the self-sampling arm was 33.5% and HPV prevalence 17.1%. In the control group 47.5% of the invited women had samples taken by midwives and HPV prevalence was 4.5%. The self-sampling approach detected a comparable proportion of high-grade dysplasia to that identified through conventional screening methods (0.48% versus 0.47%). Thus, our study indicates that self-sampling could replace primary HPV screening of cervical samples.

Paper II: The most common screening history in women with cervical cancer was irregular screening (143, 57.9%), followed by women being above screening age (44, 17.8%). Consistent participation in cervical cancer screening programmes remains the most effective strategy for prevention.

Paper III: The content analysis identified seven categories, and the overarching theme became "the HPV self-test reduced practical and emotional barriers to attending the cervical cancer screening programme, but test results may create anxiety".

Paper IV: The return rate for vaginal self-sampling kits was 37%, with an HPV prevalence of 19%. Of those requiring follow-up, 81% completed their appointments, and 39% were still HPV-positive. The proportion of biopsy-verified high-grade cervical dysplasia or cancer was 0.5% (1,012/204,763) of all eligible self-samples. The direct distribution of self-sampling kits has proven effective in facilitating HPV detection and enabling early intervention, highlighting its potential to contribute to cervical cancer prevention even in populations with initially low screening compliance.

Populärvetenskaplig sammanfattning

Livmoderhalscancer är den fjärde vanligaste cancerformen bland kvinnor globalt. Statistik från 2022 visar en incidens på 662 301 fall, varav cirka hälften avled till följd av sjukdomen. Mer än 85% av alla fall av livmoderhalscancer förekommer i låginkomstländer. I Sverige diagnostiserades 473 kvinnor med invasiv livmoderhalscancer år 2023 och 119 (25%) kvinnor avled samma år till följd av sjukdomen. Den största riskfaktorn för att utveckla livmoderhalscancer i höginkomstländer är uteblivet deltagande i organiserade screeningprogram, medan den höga incidensen i låginkomstländer framförallt kan förklaras av hög HPV-prevalens i kombination med bristande primär- och sekundärpreventiva insatser.

Humant papillomvirus (HPV) är världens vanligaste sexuellt överförbara infektion och orsakar i princip samtliga fall av livmoderhalscancer. De flesta HPV-infektioner läker ut spontant inom 1–2 år. Vid persisterande HPV-infektion tar det i genomsnitt 15–20 år att utveckla livmoderhalscancer. Idag finns effektiva verktyg för att förebygga sjukdomen. Progression till invasiv cancer kan i hög grad undvikas genom effektiv screening som möjliggör tidig upptäckt och behandling av höggradiga cellförändringar. HPV-vaccination med Gardasil-9, som skyddar mot sju högrisktyper och två lågrisktyper av HPV, förebygger cirka 90% av alla fall av livmoderhalscancer och har högst effekt om den ges före sexualdebut. Sverige har goda förutsättningar att nå Världshälsoorganisationens (WHOs) mål om att eliminera livmoderhalscancer, definierat som en incidens <4/100 000, redan till år 2027. Detta beror på den höga täckningsgraden för deltagande i det HPV-baserade screeningprogrammet, vilket uppgick till 82,9% år 2024. Skolbaserad HPV-vaccination infördes 2012 för flickor i årskurs 5 och år 2020 för pojkar i motsvarande årskurs. År 2023 var täckningsgraden för två vaccinationsdoser 87,3% för flickor och 82,2% för pojkar i skolåldern. Sedan införandet av ”catch-up”-vaccination för kvinnor födda 1994–1999 har 64,8% av dessa kvinnor både vaccinerats mot HPV och genomgått HPV-test i Sverige. Redan nu finns studier som visar att de vanligaste onkogena HPV-typerna (16 och 18), vilka orsakar närmare 90% av alla cancerfall, numera är mycket ovanliga i vaccinerade ålderskohorter.

I Sverige har kvinnor traditionellt screenats med cytologiprov sedan införandet på 1960-talet, men 2015 rekommenderades istället primär testning för HPV. Screeningprogrammet har varit effektivt, med en incidensminskning på cirka 66% och en mortalitetsreduktion på 86% sedan införandet av ett välorganiserat nationellt screeningprogram. Ett negativt HPV-test ger ungefär sju gånger högre skydd mot framtida cancer jämfört med ett negativt cytologisvar. HPV-baserad screening har varit implementerad i Region Skåne sedan 2017 och år 2021 infördes primär testning med vaginalt självprov för HPV. Fördelarna med självprovtagning är flera, bland annat minskade kostnader för hälso- och sjukvården genom effektivare

resursanvändning, samt ökat deltagande bland kvinnor som tidigare uteblivit från screeningprogrammet.

Studie I: Den primära frågeställningen i denna studie var om självprovtagningskit för HPV hörsammades bland kvinnorna som blev inbjudna att deltaga, prevalensen av HPV och andelen förstadier till cancer som verifierades genom vävnadsprover från livmoderhalstappen i självtestgruppen. Vid denna tidpunkt screenades alla kvinnor från åldern 30-64 år primärt med HPV-testning. Vi skickade således ut självprovtagningskit för HPV till 14 765 kvinnor i Region Skåne under september 2019 i åldersgrupperna 30-64 år. De valda kvinnorna skulle ha blivit kallade till det ordinarie screeningprogrammet samma höst. Provsvaren jämfördes sedan med en kontrollgrupp bestående av 14 839 kvinnor som blev kallade till sin ordinarie cellprovskontroll hos barnmorska under hösten 2019. I självtestgruppen skickade 4 943 (33,5%) kvinnor in sitt test, varav 815 (17,1%) var HPV positiva. I kontrollgruppen gick 7 042 (47,5%) kvinnor på sin barnmorskekontroll, varav 316 (4,5%) var HPV positiva. Antalet detekterade förstadier till livmoderhalscancer var 23 (0,5%) i självtestgruppen respektive 33 (0,5%) i kontrollgruppen. Självprovtagning identifierade en jämförbar andel förstadier till livmoderhalscancer som ordinarie screening (kontrollgruppen). Vår studie indikerar därför att självprovtagning skulle kunna ersätta primär HPV-screening av cervixprover.

Studie II: Denna studie är en kvalitetsgranskning. Data samlades in från det Nationella registret för prevention av livmoderhalscancer, Region Skånes Labmedicin-databas samt journalsystemet Melior under åren 2017–2020 efter att primär HPV-baserad screening införts i Region Skåne. Syftet med denna studie var att kartlägga screeninghistoriken, uppföljande kontroller samt given behandling hos kvinnor som diagnostiserats med livmoderhalscancer år 2017-2020 för att utvärdera hur väl vårt screeningprogram fungerar. Totalt sett diagnosticerades 247 kvinnor med livmoderhalscancer, varav 57,9% inte hade deltagit regelbundet på screening, 17,8% var kvinnor som nått över screeningåldern, 14,2% hade screenats enligt rekommendation och 10,1% fick sin cancerdiagnos mellan screeningintervall. HPV detekterades i 96% av alla cancerfall. Majoriteten av kvinnor som diagnosticerades med livmoderhalscancer upptäcktes pga symptom, medan screeningprogrammet hittade 38,9% och 0,8% detekterades som ett bifynd. Denna studie visar således att den viktigaste sekundärpreventiva åtgärden för livmoderhalscancer är deltagande i screeningprogrammet.

Studie III: Syftet med denna studie var att utvärdera kvinnors upplevelse av självtestning för HPV. Vi använde oss av en kvalitativ studiedesign och innehållsanalys med ett induktivt förhållningssätt. Studien riktade sig till kvinnor i screeningålder, 23-70 år, bosatta i Region Skåne och som fått ett erbjudande om självtestning sedan införandet hösten 2021. Enkätundersökningen bestod av nio öppna frågor och nio bakgrundsfrågor och ifylldes digitalt. Inbjudningar om deltagande skickades ut till 1 500 kvinnor som inte deltagit regelbundet i screeningprogrammet tidigare, 300 kvinnor som deltagit enligt rekommendationer

och till 115 kvinnor som genomgått konisering efter påvisande av HPV genom självtestet. Totalt sett svarade 173 kvinnor på enkätundersökningen. Av dessa hade 101 kvinnor deltagit regelbundet i screening, 37 kvinnor hade ej deltagit i screeningprogrammet enligt rekommendationer och 45 kvinnor hade en tidigare historik av cellförändringar. Innehållsanalysen identifierade sju kategorier: (1) obehag vid gynekologisk undersökning; (2) tacksamhet och acceptans för självprovtagning; (3) varierande uppfattning om den egna förmågan att utföra självtestning; (4) preferens för cervixprovtagning utförd av hälso- och sjukvårdspersonal; (5) oro och rädsla kopplad till en möjlig eller påvisad HPV-infektion; (6) varierande riskbedömningar avseende att smittas av en HPV-infektion; samt (7) negativ påverkan på den psykiska hälsan till följd av cellförändringar. Det övergripande temat blev: ”HPV-självtestet minskade logistiska och emotionella hinder som försvårar deltagande i screeningprogrammet för livmoderhalscancer, men testresultaten kan skapa oro.”

Studie IV: I denna studie har vi utvärderat implementeringen och utfallet av självprovtagning inom livmoderhalscancerscreening i en verklig klinisk kontext från september 2021 till och med slutet av året 2024. Data samlades in via Laboratory Information Management System (LIMS) och journalsystemet Melior. Totalt skickades det ut 557 976 vaginala självprovtagningstest och 37% av dessa kvinnor tog sitt självtest varav 19% (39 697) var HPV positiva. Bland de HPV-positiva kvinnorna deltog 81% (32 305) på uppföljande kontroll hos barnmorska inom fyra månader då ett förnyat cellprov togs. Cervixproven var HPV-positiva hos 12 495 kvinnor (39%) och HPV 16 eller HPV 18/45 påvisades i 17% av fallen. Höggradiga cellförändringar i cytologisvar identifierades bland 6,1% (759) av kvinnorna och biopsiverifierade höggradiga förändringar bekräftades i 980 fall (13%). Totalt diagnosticerades 32 (0,4%) kvinnor med livmoderhalscancer. Programmet för vaginal självprovtagning visade sig vara effektivt i att identifiera HPV-infektioner och betydande cytologiska avvikelser, vilket understryker dess potential att möjliggöra tidig upptäckt, öka screeningdeltagandet och främja snabb klinisk intervention, vilket i sin tur kan minska incidensen av livmoderhalscancer.

Sammanfattningsvis har ovan studier visat att utskickade självprovtagningstest för HPV uppnådde jämförbara detektionsnivåer av höggradig dysplasi som traditionella cervixprov tagna av barnmorskor. En kvalitetsgranskning visade att regelbundet deltagande i screeningprogrammet för livmoderhalscancer är den mest effektiva strategin för sekundärprevention. Kvalitativa studier och implementeringsstudier fann att självprovtagning minskar barriärer för deltagande och underlättar tidig HPV-detektion, vilket gör det till en lovande strategi för att öka deltagandet i screeningen och förebygga livmoderhalscancer.

Abbreviations

AGC	Atypical glandular cells
AIS	Adenocarcinoma in situ
ASC-H	Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesions
ASCUS	Atypical squamous cells of undetermined significance
CI	Confidence interval
CIN	Cervical squamous Intraepithelial Neoplasia
CIS	Carcinoma in situ
FIGO	International federation of gynaecology and obstetrics
HPV	Human papillomavirus
hrHPV	High-risk HPV
HSIL	High-grade squamous intraepithelial lesion
IARC	International agency for research on cancer
LBC	Liquid-based cytology
LMICs	Low- and middle-income countries
LSIL	Low-grade squamous intraepithelial lesion
PCR	Polymerase chain reaction
SCJ	Squamocolumnar junction
WHO	World health organization

Introduction

Cervical cancer

Cervical cancer is the fourth most common cancer among women worldwide (1). In 2022 an estimated 662,301 new cases and 348,874 deaths were reported globally (2). The global distribution of cervical cancer is highly unequal; in Sweden, the lifetime risk is approximately 0.5%, whereas in some African countries, such as Eswatini, it reaches around 7% (3), and is the leading cause of cancer death among women (1). This disparity can partly be explained by differences in the prevalence of human papillomavirus (HPV) prevalence—the necessary cause of cervical cancer—which is substantially higher in certain regions (4), and secondly by the implementation of effective primary and secondary preventive strategies. HPV vaccination protects against infection and reduces transmission, and organised cervical cancer screening programmes enables early detection and disease control (5).

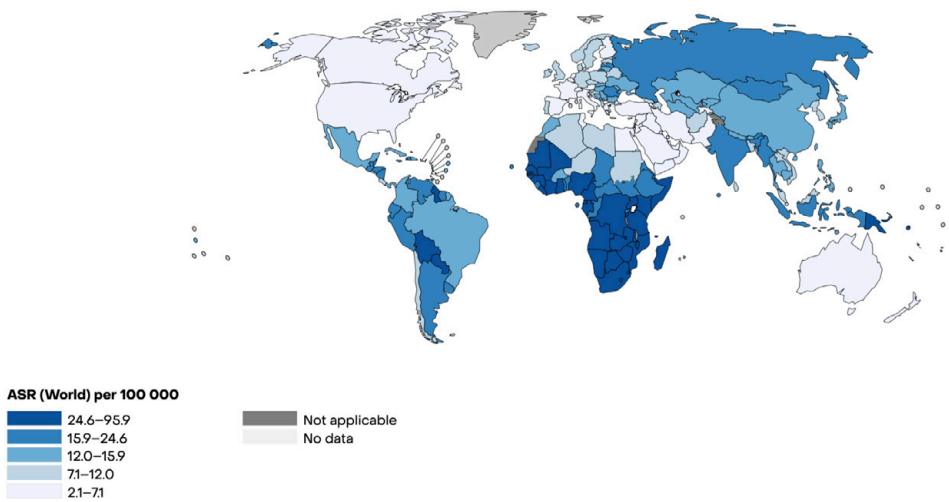

Figure 1. Age-standardized incidence rates (World) per 100 000, all females, cervix uteri, 2022. Data source GLOBOCAN 2022. Graph production: IARC https://gco.iarc.who.int/today World Health Organization.

Cytological smears have served as the cornerstone of cervical cancer screening in Sweden since their introduction in the 1960s (6). In recent years, however, vaginal HPV self-sampling has been introduced as an alternative approach (7). The national screening programme has been associated with substantial effectiveness, and estimates indicate a 66% reduction in incidence and an 86% reduction in mortality since the introduction of the screening programme in Sweden (8).

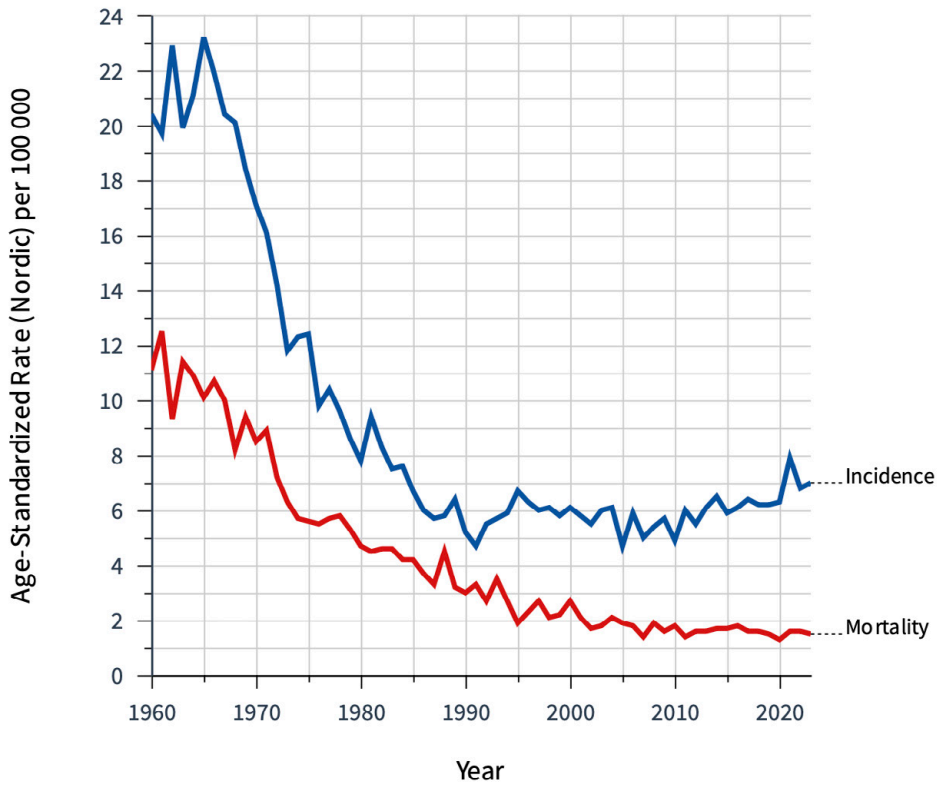

Figure 2. A decline in cervical cancer incidence and mortality rates in Sweden since the introduction of an organised screening programme. Age-standardized rates (Nordic) per 100 000 from 1960-2023. Data source: NORDCAN https://gco.iarc.who.int/en.

Public health perspective

Screening serves as an initial filtering process applied to a healthy, asymptomatic population, and is designed to separate individuals with a higher probability of having the condition while excluding those with a lower probability. The primary goal of the cervical cancer screening programme is to detect and treat premalignant lesions, thereby reducing the incidence and mortality associated with the disease (9). Cervical cancer is a potentially fatal disease, yet it is highly preventable through HPV vaccination and effective screening programmes (5). It is widely recognised that HPV infection causes almost all cervical cancer cases (10). Progress to invasive cervical cancer usually evolves slowly, with predefined premalignant lesions, which enables the screening programme to efficiently detect and treat dysplastic lesions before the onset of cervical cancer (11). Accumulated evidence has shown that HPV testing demonstrates higher sensitivity in the detection of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) compared to cytology testing, and HPV testing is now the cornerstone of cervical cancer screening programmes in Sweden (12). Screening with HPV self-sampling has also shown high acceptability among women (13) and eliminates practical and emotional barriers for attendance, thus increasing the coverage ratio and detection of early precancerous lesions (14-16). The majority of all cervical cancer cases in high-income countries like Sweden occur among under-screened women (17), who are also more likely to receive a diagnosis at a later stage of the disease (18). Comparisons of various strategies to reach non-attenders—such as invitation or reminder letters, opt-in approaches, and direct mailing of self-sampling kits—indicate that the most effective method for increasing screening uptake is the direct distribution of self-sampling kits to women's home addresses (19). HPV self-sampling is also more cost-effective than pap smear screening due to lower material and testing costs, as well as the strategy of inviting only HPV-positive women for follow-up appointments. This approach increases overall screening participation by reaching a higher proportion of non-attenders and benefits from the higher sensitivity of HPV testing in detecting CIN2+ lesions compared to cytology (20).

In order to reduce the cervical cancer incidence to below less than 4/100,000 cervical cancer cases yearly, which is considered by the World Health Organization (WHO) to be a low incidence, a combination of primary and secondary preventive measures must continue to be evaluated and improved (21). The WHO's cervical cancer elimination initiative, launched in 2020, aims to accelerate the elimination of the disease through three key targets: 1) achieving 90% HPV vaccination coverage among girls by the age of 15, 2) ensuring that 70% of women undergo at least two HPV screening tests in their lifetime (one before the age of 35 and one before the age of 45), and 3) providing treatment for 90% of women diagnosed with premalignant lesions and 90% of those with invasive cervical cancer. These goals have the potential to make cervical cancer a rare disease in the future, with fewer

than four cases per 100,000 women per year (22). In Sweden, a "catch-up" vaccination with concomitant HPV testing for women born in the period 1994-1999 was introduced in Sweden in 2021 aiming for an even faster elimination of cervical cancer (23).

In 1968, Wilson and Jungner introduced 10 principles aimed at ensuring the clinical effectiveness of screening programmes. In summary, these principles emphasise that the condition should represent a significant public health concern with a well-understood natural history. Effective diagnostic and treatment services must be accessible, with clearly defined criteria for treatment. The disease should have a detectable early stage, and a reliable, acceptable screening test should be available. Ultimately, the screening programme must be cost-effective and implemented as a continuous, systematic process subject to regular evaluation (24). In Sweden, the National Board of Health and Welfare has expanded these principles into 15 criteria that must be met for the cervical cancer screening programme. While the original 10 principles, which primarily focus on clinical effectiveness, remain integral, five additional criteria were introduced in 2014 to better align with the Swedish healthcare system. These additional criteria emphasise ethical considerations, equity, accessibility, and cost-effectiveness (25). The cervical cancer screening programme in Sweden has proved effective; however, similar improvements have not been observed in low- and middle-income countries (LMICs) (26). The burden of cervical cancer remains unevenly distributed across geographic regions and socio-economic groups globally, with over 85% of cases occurring in low-income countries. In high-resource countries, both the incidence and mortality rates are two to four times lower (5). Furthermore, women diagnosed with HIV face a sixfold increased risk of developing cervical cancer compared to HIV-negative women (27). Contributing to the limited success of preventive efforts in many low-resource settings are inadequate infrastructure and restricted access to technical equipment and trained healthcare professionals (28). In LMICs, HPV testing is considered the most effective cervical cancer screening method, with modelling studies estimating a 63–67% reduction in mortality when screening is conducted every five years and achieves a coverage rate of 70% (29). Screening programmes must be continuously evaluated and effectively adapted to national conditions and healthcare infrastructures, with the overarching aim of contributing to the WHO's goal of eliminating cervical cancer in the near future.

Identifying an ideal screening test that balances both sensitivity and specificity is inherently complex. Given that no screening test is perfectly sensitive or specific, false-positive and false-negative results are inevitable. High sensitivity enhances the negative predictive value (NPV), providing greater reassurance to women who test HPV-negative and supporting the extension of screening intervals. Moreover, increased sensitivity facilitates the early detection and treatment of precancerous lesions, thereby reducing the morbidity and mortality associated with cervical cancer. Conversely, high clinical specificity improves the positive predictive value

(PPV), minimising false-positive results (30). A lower specificity may consequently lead to overdiagnosis and overtreatment, contributing to psychological distress, and increased healthcare expenditure (9). The benefits of a screening programme must be maximised while ensuring that any potential harm is outweighed. A comparative analysis of the more intensive cervical cancer screening programme in the United States and the less intensive programme in the Netherlands demonstrated a two- to threefold increase in adverse outcomes—such as preterm birth, anxiety, pain, and bleeding—among the American population. Notably, the incidence and mortality rates of cervical cancer remained comparable between the two countries (31). Long-term data demonstrated no significant difference in the cumulative incidence of CIN2+ between HPV-based screening and cytology, indicating that the superior sensitivity of HPV testing is probably due to earlier detection rather than overdiagnosis (32). However, gains in sensitivity may come at the expense of reduced specificity (9). In the cervical cancer screening programme, a high sensitivity threshold is prioritised to minimise the likelihood of false-negative results, ensuring that very few cases of disease are missed among screened women. For self-sampling, maintaining high sensitivity is particularly important, as more non-attenders—who may only participate in screening via self-sampling—rely on this as their sole opportunity for early detection. Consequently, follow-up triage of HPV-positive women is essential to distinguish transient infections from those with a higher risk of progression to CIN2+ lesions. Additional molecular or cytological assessments during follow-up can enhance specificity (12, 33-36).

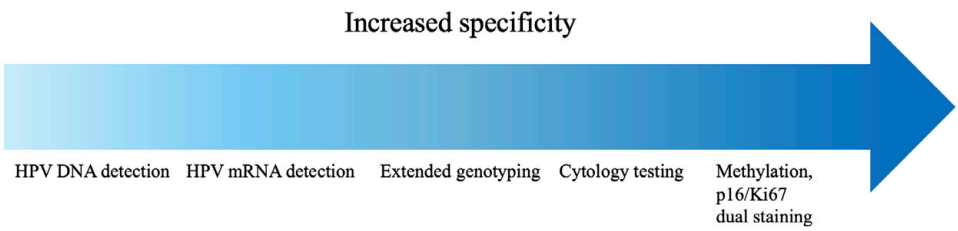

Figure 3. Various screening modalities and their respective impact on specificity.

Human papillomavirus (HPV)

HPV biology

The HPV genome is a circular, double-stranded DNA molecule comprising approximately 8,000 base pairs. It is organised into three primary regions: the early (E) region, the late (L) region, and the long control region (LCR). The early region, which includes the E1, E2, E4, E5, E6, and E7 genes, encodes proteins that are essential for the viral life cycle and are critically involved in cellular transformation. The late region, consisting of the L1 and L2 genes, encodes the structural proteins that form the viral capsid. Lastly, the LCR functions as a regulatory domain, controlling viral gene expression and replication (37, 38). HPV DNA testing identifies the virus by targeting its genetic material, whereas HPV mRNA assays detect the oncoproteins E6 and E7. The presence of E6/E7 mRNA reflects active viral replication and is more closely associated with the progression toward precancerous lesions; therefore, it can offer greater specificity than DNA-based methods (22).

HPV life cycle

Microtrauma to the cervical epithelium facilitates the entry of HPV virions into the basal cell layer in stratified epithelium. In columnar cell layers, infection may happen more easily because the target cells lie close to the surface (39). Once inside the host cells, viral DNA replication is initiated by the early proteins E1 and E2. The viral oncoproteins E6 and E7 are then expressed; E6 binds to and promotes the degradation of p53, thereby preventing apoptosis, while E7 inactivates pRB, effectively removing cell-cycle arrest. In the differentiated upper epithelial layers, the late proteins L1 and L2 are synthesised, enabling encapsidation of the amplified viral genomes. Mature virions are subsequently released from the epithelial surface, completing the productive phase of infection (10, 37, 40).

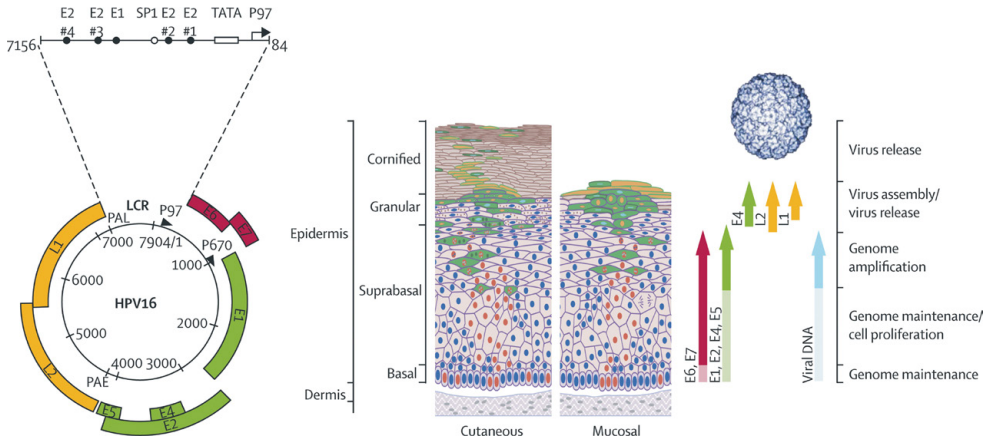

Figure 4. The HPV genome and the life cycle of HPV within the cervical epithelium. Reprinted from The Lancet, Volume 370, Issue 9590, 890 – 907, Schiffman, Mark et al., "Human papillomavirus and cervical cancer", 2007, with permission from Elsevier.

HPV infections are, in most cases, eliminated through the activation of cell-mediated immune responses. When the viral genome is suppressed, it remains in the basal epithelial layer without inducing any clinically detectable infection, thereby constituting a latent infection. Low-grade Squamous Intraepithelial Lesion (LSIL) is characterised by a regulated expression of viral genes, whereas High-grade Squamous Intraepithelial Lesion (HSIL) reflects a dysregulated pattern of viral gene expression. The accumulation of secondary genetic alterations in infected host cells, driven by the overexpression of the E6 and E7 oncoproteins, can contribute to the progression toward cervical cancer. In cases of cervical cancer, the viral genome is frequently integrated into the host cell's chromosome (39).

HPV genotypes

More than 200 HPV genotypes have been identified, of which approximately 40 are transmitted through sexual contact (41). The International Agency for Research on Cancer (IARC) classifies HPV types into three categories based on their causal association with cervical cancer: Group 1 (carcinogenic to humans), Group 2A (probably carcinogenic to humans), and Group 2B (possibly carcinogenic to humans). Among the 12 HPV types classified as carcinogenic (Group 1), HPV16 accounts for 62.4% of cervical cancer cases, HPV18 for 15.3%, and HPV45 for 4.8% (42). HPV16 is the principal etiological agent in squamous cell carcinoma, whereas for adenocarcinomas, HPV18 and HPV45 are more frequently detected (43).

Given the well-established variations in oncogenic potential among different HPV types and their associated risks for progression to invasive cervical cancer, Swedish

national guidelines recommend risk-stratified screening. This approach incorporates triage based on oncogenic classification, categorising HPV types as high-oncogenic (16, 18, 45), mid-oncogenic (31, 33, 52, 58), or low-oncogenic (35, 39, 51, 56, 59, 68) to optimise screening efficacy (44). All 13 HPV types included in these categories are classified as Group 1 (carcinogenic to humans) by the IARC, except for HPV68, which is classified as Group 2A (probably carcinogenic to humans) (44).

Most HPV infections clear spontaneously within two years (45). However, as high-risk viral types such as HPV16 and HPV18 are most strongly associated with malignant transformation, national guidelines recommend that women with these HPV infections, irrespective of cytology findings, should be promptly referred for evaluation at a colposcopy clinic (46).

The screening effort needed to avert a cervical cancer case attributable to HPV16 is markedly lower than that required for low-oncogenic HPV types, with a more pronounced disparity observed in women under 30 years of age than in those aged 51–60 years. In women younger than 30 years, the number needed to screen and the number needing follow-up were approximately 50–60 times lower for HPV16 compared with the low-oncogenic group. Among women aged 51–60 years, the corresponding difference was reduced to roughly 10–20 times (47).

Epidemiology

HPV infection is highly prevalent, and most sexually active women are expected to acquire the virus at least once during their lifetime. Most infections, however, are transient and resolve spontaneously without causing any precancerous lesions (48). Globally, the prevalence of HPV is estimated at approximately 10%, although considerable geographical variation is observed, with rates ranging from 20–30% in Africa and Latin America to 6–7% in Southern Europe and Southeast Asia (4).

An incident HPV infection is defined as an HPV-positive test obtained following a prior HPV-negative test (49), whereas a persistent HPV infection is defined as the same HPV type detected in two consecutive screening tests. A prerequisite for cervical dysplasia and subsequent cancer is a persistent HPV infection; therefore, separating transient infections from persistent HPV infections and developing an efficient screening algorithm in the triage of HPV-positive women is important to reduce the risk of overdiagnosis and over-referrals (50).

HPV prevalence typically peaks among women younger than 25 years, which is largely attributable to the onset of sexual activity (51). In some countries, a second peak has also been observed in women over 45 years of age (52). The second peak in age-specific HPV prevalence may be attributed to the reactivation of latent infections in middle-aged and older women, potentially influenced by declining sexual activity and age-related immune senescence (53).

A significant decline in the prevalence of HPV16 and HPV18 has been observed in vaccinated birth cohorts compared to pre-vaccination cohorts (54). However, the prevalence of HPV types not covered by the vaccine has remained stable across different age groups (23). HPV testing offers superior long-term protection against cervical cancer compared to cytology. The incidence of cervical cancer among women with a negative HPV test has been reported at 1.3 cases per 100,000 person-years, in contrast to 9.1 cases per 100,000 person-years among those with normal cytology results. Notably, women who test positive for HPV but have normal cytology at triage exhibit a substantially elevated cancer risk of 79.2 cases per 100,000 person-years (35).

Risk factors for HPV persistence

The risk of oncogenic transformation varies significantly depending on the HPV genotype, with odds ratios ranging from 0.3 for HPV70 to 47.6 for HPV16 (42). An elevated HPV viral load has also been observed in both low-grade and high-grade cervical dysplasia compared to cases with normal cytology (55). Several other cofactors contribute to the persistence of HPV infection and thus increase the risk for cervical disease. Concurrent sexually transmitted infections like *Chlamydia trachomatis* and herpes simplex virus type 2, may promote cervical inflammation, while HIV is known to cause chronic immune activation and to impair the body's ability to eliminate HPV. Additionally, women undergoing immunosuppressive therapy often exhibit a reduced capacity to clear HPV effectively. Individual genetic variations, such as polymorphisms in the human leukocyte antigen genes—which are essential for regulating the immune response—can affect the body's capacity to eliminate an HPV infection (56). An increased number of full-term pregnancies has been associated with a heightened risk of HPV persistence, potentially due to the sustained presence of the transformation zone on the exocervix and the immunomodulatory effects of elevated oestrogen and progesterone levels during pregnancy. Smoking and long-term use of oral contraceptives have also been shown to be associated with HPV persistence (57). Early initiation of sexual activity and a higher number of sexual partners are also associated with increased HPV persistence, primarily due to the increased likelihood of repeated exposure to the virus (58). Lastly, the vaginal microbiome, including alterations such as bacterial vaginosis, has also been proposed to influence the clearance of an HPV infection, although further research is warranted (38).

HPV transmission

Almost all cases of cervical cancer are attributable to an HPV infection. HPV is also implicated in other malignancies, including penile cancer (50%), anal cancer (88%), oropharyngeal cancer (31%), vaginal cancer (78%), and vulvar cancer (25%).

However, in these cancer types, the proportion of cases attributable to HPV is considerably lower than in cervical cancer, reflecting a more heterogeneous aetiology (59). The cervical tropism of HPV is primarily attributed to the transformation zone, a region that is especially vulnerable to infection due to the replacement of columnar epithelium with newly formed metaplastic squamous epithelium (60). The highest rates of HPV transmission occur via genital-to-genital contact, most frequently from female-to-male. Transmission via hand-to-hand contact is exceedingly rare, and genital-to-hand or hand-to-genital transmission events are also considered uncommon (61). Furthermore, the vagina and anus may serve as HPV reservoirs for autoinoculation to the cervix, while the cervix itself may act as a potential HPV reservoir for the anus (45).

HPV analytes

Validation of HPV assays

The prevalence of cervical cancer in the general population is low, and most high-risk HPV infections are destined to resolve spontaneously within 2 years, although clearance varies depending on the specific HPV type (37). The primary objective of a screening test is to identify women with oncogenic HPV types that have the potential to progress to cervical neoplasia and, ultimately, cervical cancer. Robust evidence has demonstrated that HPV testing has higher sensitivity than cytology testing alone (12), thus yielding better cancer protection and enabling longer screening intervals compared to cytology-based screening (62). To ensure that new HPV tests achieve high diagnostic quality, with an optimal balance between sensitivity and specificity for clinical use, they must align with international guidelines. The first-generation comparator tests, namely Hybrid Capture 2 (HC2) and GP5+/6+- polymerase chain reaction (PCR), have consistently proved to achieve higher sensitivity for CIN2+ in longitudinal screening trials compared to cytology. The validation protocol by Meijer et al. includes non-inferiority testing for specificity and sensitivity in detecting CIN2+, with relative thresholds set at 98% and 90% respectively, compared to the first-generation comparator test, as well as standards for intra- and interlaboratory reproducibility (30). Similar to the Meijer protocol, the VALGENT (VALidation of HPV GENoyping Tests) framework aims to evaluate new HPV assays for non-inferiority compared to standard comparator tests, ensuring their quality assessment for clinical use in cervical cancer screening programmes. However, it differs in that a panel of cervical samples is obtained from a screening population using stored samples and extends to HPV assays that offer genotyping capabilities (63).

In 2023, more than 250 different HPV assays were available in the global market (64), but the updated list of validated HPV assays on cervical samples published in

2024 presents only 19 HPV DNA assays and one HPV mRNA assay (Aptima) that fulfil the criteria for use in clinical settings (65). All 12 HPV types classified as carcinogenic by the IARC are covered by these 20 validated HPV assays (42). However, the validated HPV assays vary in their genotyping capacities, with only six offering full genotyping capacity (65). It is well-established that different hrHPV types vary in their oncogenic potential (42). Therefore, it is crucial for future HPV assays to incorporate this differentiation. The Swedish national guidelines recommend classification and triage based on oncogenic risk categories: high-oncogenic (16, 18, 45), mid-oncogenic (31, 33, 52, 58), and low-oncogenic (35, 39, 51, 56, 59, 68) (44). HC2 and GP5+/6+ are no longer widely used in laboratories, as emerging HPV assays have entered the market (66). Consequently, new criteria have been established for second-generation comparator tests. Among these, four HPV assays fulfilled the criteria to serve as standard comparator tests: the Real-Time High-Risk HPV Test, the Cobas-4800 HPV test, the Onclarity HPV Assay, and Anyplex II HPV HR Detection (67).

The Aptima mRNA assay

Performance of the Aptima mRNA assay

The majority of HPV assays on the market are based on the detection of DNA (65). However, in the southern part of Sweden, an mRNA-based assay, the Aptima (Hologic, San Diego, CA, USA), has been used in the screening programme since January 2017. The Aptima mRNA assay is also implemented in Örebro, a city further north in Sweden (68), in Wales, the Basque Country (Spain), and Scotland (66). This mRNA assay was validated for clinical use in 2013 by Heideman et al., and demonstrated a clinical sensitivity of 94.2% and a specificity of 94.5% for detecting CIN2+ (69). Further quality controls of the Aptima mRNA assay have been conducted subsequently, showing similar sensitivity for the detection of CIN2+ but slightly higher specificity compared to other gold standard DNA-based assays (33, 70, 71). This assay targets viral mRNA from the E6/E7 oncogenes. Overexpression of E6/E7 mRNA transcripts could explain the increased specificity, as transcriptionally active infections are more likely to progress to cervical dysplasia (33).

Since validation criteria are based on DNA detection of HPV, limited studies exist regarding longitudinal outcomes for the protective effect of a negative HPV mRNA test (66). Two previous studies have demonstrated the long-term safety of extending cervical cancer screening intervals to 5-7 years when using the Aptima mRNA assay. In these studies, the Aptima showed comparable longitudinal performance to two DNA-based assays (HC2 and Cobas 4800), suggesting a minimal risk of CIN3+ development following a negative HPV test result. However, current WHO guidelines recommend an mRNA-based screening interval with cervical samples of

no more than five years, since the accumulated evidence is heterogeneous and limited. Further research is therefore needed to establish the long-term safety of extending screening intervals beyond five years (22).

The Aptima detects 14 hrHPV types from the E6/E7 mRNA (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 82), three potentially hrHPV types (26, 66, 67), and one low-risk type (70). For self-collected samples, a cotton swab is used to collect cells from the vagina by inserting it 5 cm into the vagina and rotating it 360°, two or three times. The swab is then placed into a sample tube containing 2.9 ml of STM, which lyses the cells, releases mRNA, and prevents its degradation during transport. Cervical samples are collected by healthcare professionals using a cytobrush to obtain cells from the cervix, which are then transferred into 20 mL of PreservCyt (Hologic, Bedford, MA, USA). Notably, only self-collected samples undergo a pre-heating step at 90°C for 1 hour and 15 minutes prior to analysis. The analytic process is then divided into three main steps: 1) Capture of E6/E7 mRNA coding for oncoproteins using DNA probes conjugated to magnetic microparticles; 2) Amplification of RNA using transcription-based nucleic acid amplification (TMA), which is an isothermal process; and 3) Detection of RNA fragments using probes that emit light. The emitted light is measured as Relative Light Units in a luminometer. The analyser interprets the signal-to-cutoff (S/CO) ratio, classifying the result as positive, negative, or invalid.

There are some limitations to the Aptima HPV mRNA assay. First, there is no internal control for human genes as in most other HPV assays. This may cause false negatives as the assay does not confirm sample adequacy. Nevertheless, the Aptima incorporates an internal control that ensures the presence of nucleic acids and monitors the amplification and detection steps of the assay (72). Second, the genotyping capacity is restricted as it only separates the high-risk HPV types 16, and combines the detection of HPV18 and 45. This unables further triage of HPV-positive women according to the high-, mid- and low-oncogenic HPV types recommended by the national guidelines. Subsequently, screening with the Aptima mRNA assay will be discontinued in southern Sweden, and procurement of a new assay is currently in progress, with implementation anticipated during autumn 2025.

Self-sampling with the Aptima mRNA assay

Self-sampling has been found to be as reliable and accurate as clinician-collected samples for HPV detection when based on PCR (73). The Aptima mRNA assay is not based on PCR; instead, it uses transcription-mediated amplification, a nucleic acid amplification technology that amplifies RNA rather than DNA (72). A meta-analysis by Arbyn et al. from 2022 showed comparable specificity but slightly lower sensitivity (relative sensitivity 0.84 [95% confidence intervals (CI) 0.74–0.96]) to detect CIN2+ on self-samples when using the Aptima mRNA assay compared to DNA-based assays on cervical samples (66). Since endocervical cells serve as the

primary site for high-risk HPV infection and exhibit the highest viral load compared to the vagina, a highly sensitive test is necessary to ensure comparable detection accuracy between clinician-obtained and self-collected samples (74). In the southern part of Sweden, the clinical sensitivity of the Aptima assay has been improved by pre-heating vaginal self-samples for 75 minutes before analysis, increasing the sensitivity from 85.3 to 95.3% (75). The introduction of a pre-heating step before analysis has been shown to enhance sensitivity; however, this comes at the expense of reduced specificity, as approximately two-thirds of women do not have a detectable HPV infection in the follow-up clinician-collected cervical sample in our two previous studies (76). The primary task of the Aptima mRNA assay is to detect E6/E7 mRNA transcripts of HPV-infected cells; however, HPV DNA can also be detected (77). In PCR-based methods, DNA is separated into single-stranded templates at temperatures around 95°C (78), whereas mRNA is a less stable molecule and starts degrading at temperatures around 40°C (79). The pre-heating step in our method probably results in an increased number of single-stranded HPV DNA molecules, which are then captured with the Aptima. This step could also help to break down any secondary structures or proteins that might be binding to the mRNA, making it more accessible for detection. However, more research is warranted to validate the mRNA assay on self-samples and the effect of the pre-heating step (66). This research should be performed according to the VALHUDES (VALidation of HUman papillomavirus assays and collection DEvices for HPV testing on Self-samples and urine samples) protocol (80).

Factors influencing the self-sampling procedure

Factors that influence the analytical performance of HPV testing include the collection device and sampling instructions, whether dry or wet samples are used, the transport medium and the resuspension volume, the storage conditions and stability of the sample, the pre-analytical procedure, and the different HPV assays and their cut-offs for detection rate (81). Consistent performance can only be achieved if the self-collected specimen is properly obtained and stored during transport before detection using a validated HPV assay. Such assays and collection procedures should be validated in accordance with the VALHUDES protocol, which provides a standardised framework for the clinical validation of HPV testing methods and collection devices used with vaginal self-samples and urine samples (80).

A study conducted in the Netherlands reported higher PCR cycle threshold (Ct) values in self-collected vaginal samples compared to clinician-collected cervical samples, indicating lower viral loads in the vaginal specimens. The self-samples demonstrated a 6% lower sensitivity for detecting CIN3+ and a 2% higher specificity relative to clinician-collected samples. The authors proposed that the reduced viral concentration in self-collected samples may be partly explained by the 20 mL resuspension volume used with the Evalyn Brush device, potentially leading

to dilution of cellular material (82). Improved sensitivity of vaginal self-samples was reported when resuspension was carried out in 2.5 mL rather than 20 mL, a difference attributed to the likely increase in cellular concentration (83). In Region Skåne, the Hologic Multitest Swab Specimen Collection Kit is used, where a cotton swab is transferred into 2.9 mL of Swab Transport Media (STM).

HPV vaccination

The bivalent and quadrivalent vaccines both target hrHPV type 16 and 18, responsible for approximately 70% of all cervical cancer cases globally (84). The nonavalent vaccine targets seven hrHPV types (16, 18, 31, 33, 45, 52, 58), and these hrHPV types are detected in about 90% of all cervical cancer cases (85). Furthermore, the quadrivalent vaccine has shown 98% efficacy in preventing HPV16 and 18-related CIN2+ and Adenocarcinoma *in situ* (AIS) (86). In a Swedish cohort of females aged 10–30 years followed over 11 years, those who received the quadrivalent HPV vaccine had an 88% lower incidence of cervical cancer when vaccinated before the age of 17, and a 53% reduction when vaccinated between the ages of 17 and 30, compared with the unvaccinated cohort (87).

School-based HPV vaccination for girls in grades 5 to 6 was introduced in Sweden in 2012 (88), and a gender-neutral vaccination approach was implemented in 2020 (54). The quadrivalent vaccine was given in a three-dose schedule until 2015, when a two-dose schedule was implemented instead (87). In 2019, the nonavalent vaccine replaced the quadrivalent vaccine (88). To achieve an even faster elimination of cervical cancer, a catch-up vaccination with concomitant HPV testing for women born 1994-1999 was introduced in Sweden in 2021, aiming for an even faster elimination of cervical cancer (23).

In the post-vaccination era, it has been proposed that screening for hrHPV types should be limited to the seven hrHPV types covered by the nonavalent vaccine to improve the efficiency of screening programmes (89). Prioritising the detection of HPV types with the highest oncogenic potential allows for a more effective allocation of healthcare resources, ensuring that efforts are concentrated where they yield the most significant public health benefits. The inclusion of HPV genotypes associated with only a small percentage of invasive cervical cancer decreases the predictive value of screening, thereby reducing its efficiency and cost-effectiveness. This targeted approach enhances the effectiveness of screening programmes while minimising the risks of overdiagnosis and unnecessary follow-up procedures (90).

The cervix

Anatomy

The cervix is anatomically divided into two main regions: the lower portion, which projects into the vaginal canal (ectocervix), and the upper portion, situated above the vagina and extending to the uterine isthmus (endocervix). It typically measures approximately 4 cm in length and 3 cm in diameter. It is covered by two types of epithelia: squamous and columnar. The endocervical canal is lined by glandular (columnar) epithelium, whereas the ectocervix and the vaginal surface are covered by squamous epithelium (60).

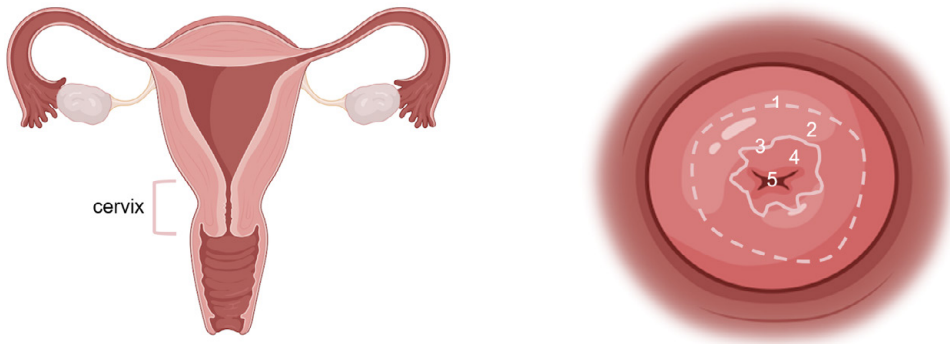

Figure 5. Anatomy of the female reproductive system with a magnified view of the cervix (right). Numbered labels indicate: (1) original squamocolumnar junction (SCJ); (2) transformation zone with metaplastic squamous epithelium; (3) new squamocolumnar junction; (4) columnar epithelium; (5) external os. Created in BioRender. Hellsten, C. (2025) https://BioRender.com/1937nrc

As epithelial cells differentiate and migrate from the basal layer toward the superficial layer, they undergo an increase in cytoplasmic volume accompanied by a progressive reduction in nuclear size. This maturation process leads to a transition from basal and parabasal cells to polygonal intermediate cells, and ultimately to large, flattened superficial cells characterised by condensed and shrunken nuclei. On examination, the squamous epithelium appears smooth and pale pink in colour. The maturation and glycogenation of cervical squamous epithelium are dependent on oestrogen. Following menopause, decreased oestrogen levels impair epithelial maturation, leading to the development of a thin, atrophic epithelium. The endocervical canal is lined by a single layer of tall columnar (glandular) epithelium with basal nuclei. Unlike the thicker stratified squamous epithelium, it appears reddish on examination due to the translucency of the thin epithelial layer and exhibits a characteristic grape-like appearance resulting from mucosal folds and crypts (91).

The squamocolumnar junction (SCJ) represents the interface between squamous and glandular epithelium. Its location is dynamic and shifts throughout life, influenced by factors such as age, hormonal status, childbirth trauma, oral contraceptive use, and pregnancy. In prepubertal girls, the original SCJ is located within the endocervical canal. Following puberty, oestrogen induces eversion of the columnar epithelium onto the ectocervix. Exposure of this epithelium to the acidic vaginal environment leads to its replacement by metaplastic squamous epithelium. This process begins at the original SCJ and results in the formation of a new SCJ, with the region between the two referred to as the transformation zone, where almost all cervical cancers originate (91).

Colposcopic evaluation

When assessing the colposcopic appearance, the use of the "Swede score" is recommended. This scoring system evaluates five parameters: acetowhitening, lesion margins, vascular pattern, lesion size, and iodine uptake. Each parameter is scored from 0 to 2 points. A total score of 8–10 suggests HSIL, whereas a score of 4 or less argues against HSIL. The transformation zone (TZ) is also classified into three types based on its anatomical location and visibility. Type 1 TZ is entirely located on the ectocervix. Type 2 TZ extends partially into the endocervical canal, but the SCJ remains fully visible. Type 3 TZ also extends partially into the endocervical canal; however, the SCJ is not visible (46).

Mature squamous epithelium contains abundant cytoplasmic glycogen, which stains a mahogany-brown colour when treated with Lugol's iodine. In contrast, immature squamous epithelium does not exhibit this staining pattern due to the absence of glycogen production. Similarly, columnar epithelium lacks intracellular glycogen and therefore does not take up Lugol's iodine. Acetic acid acts by dehydrating epithelial cells and inducing reversible coagulation of nuclear proteins. As a result, areas exhibiting cervical intraepithelial neoplasia (CIN), characterised by increased nuclear activity, undergo acetowhitening. The degree of whitening varies with lesion severity, appearing as a faint milky or translucent shade in low-grade lesions and as a dense, opaque white in high-grade lesions.

Treatment of precancerous lesions

Cervical cancer typically develops over a long period of time, approximately 15-20 years from initial HPV infection to malignant transformation. Therefore, screening plays a critical role in the early detection and treatment of precancerous lesions, effectively preventing the onset of invasive cervical cancer (92). High-grade cervical dysplasia (CIN2–3) is preferably managed by surgical excision (conisation) to prevent progression to cervical cancer (46). However, this approach is not recommended for all age groups. To reduce overtreatment and minimise the harms

of intervention—particularly in younger women, among whom CIN2 is common, often regresses spontaneously, and rarely progresses to cervical cancer (93, 94) — national guidelines advise active surveillance in specific cases. Women ≤ 25 years of age with HSIL (CIN2) and hrHPV types 16, 18, or 45, as well as women aged 23–29 with mid-oncogenic HPV types, are recommended to receive a follow-up with repeat colposcopy after six months (46).

The rate of progression from CIN to invasive cancer does not differ between pregnant and non-pregnant women (95, 96). Furthermore, statistically significant higher regression rates (56.9% versus 31.4%) and lower persistence rates (39.2% versus 58.8%) have been observed among pregnant women diagnosed with CIN1-3 compared to non-pregnant women (96). Women diagnosed with cervical cancer should receive treatment during pregnancy; however, if HSIL is detected, treatment may be safely postponed until the postpartum period to minimise the risk of treatment-related complications (95, 96).

In contrast, LSIL (CIN1) is generally managed conservatively with active surveillance since spontaneous regression is seen in about 60% of cases, whereas for CIN2, regression is seen in 55% and for CIN3, in 28%. Progression to CIN2+ and CIN3+ occurs in about 11% and 2% of cases, respectively. For CIN2, progression to CIN3 is observed in approximately 19% of cases. Progression to invasive cervical cancer is reported in about 2% of cases in women with CIN3 (97). A well-known and ethically controversial study conducted in New Zealand revealed that 31% of women with untreated CIN3, confirmed by histopathology, progressed to cervical cancer over three decades (98). Another study found that among patients with histopathologically confirmed CIN2+ undergoing active surveillance, 62.9% of lesions regressed after 24 months, while 33.3% progressed to CIN3+ (99).

Complications of conisation include bleeding, infection, preterm birth, and cervical stenosis (46). Women with a cone length >10 mm have an increased risk of preterm birth, rising by 15% per additional millimetre, while those with a cone length ≤10 mm show a 50% higher risk compared with women with normal cytology or untreated CIN (100). An elevated risk of preterm birth has also been demonstrated among women with untreated cervical dysplasia during pregnancy. In a population-based study from Western Sweden, the relative risk was 30% higher compared with women with normal cytology (101). Ultimately, women with a history of treatment for CIN3 have been shown to have a more than two-fold increased long-term increased risk of cervical or vaginal cancer. This risk escalates with advancing age, particularly beyond 60 years (102).

Cervical cancer screening

The screening programme in Sweden

A nationally organised cervical cancer screening programme was introduced in Sweden in the late 1960s. Cytology-based screening has traditionally formed the foundation of this programme. However, in 1983, Harald Zur Hausen identified the causal role of HPV in the development of cervical cancer, a discovery for which he was awarded the Nobel Prize in 2008. His findings paved the way for the development of HPV vaccines, and the first HPV test received Food and Drug Administration approval in 1988. Despite this, it took nearly three decades before Swedish guidelines recommended primary HPV testing. In the southern part of Sweden, primary-based HPV screening was implemented in January 2017 for women above 30 years of age, since this has higher sensitivity than cytology alone (12). For women under 30, cytology-based screening remained the standard due to the high prevalence of transient HPV infections in this age group. HPV testing in this population risked overdiagnosis, potentially causing unnecessary anxiety and referrals for colposcopy (103). However, in the post-vaccination era, most women entering the screening programme at the age of 23 have received the HPV vaccine, leading to reduced prevalences of high-risk HPV associated with cervical dysplasia (104).

In September 2021, HPV self-sampling was introduced as the primary screening method in Region Skåne. In both Region Skåne and Örebro, HPV analysis is conducted using the Aptima mRNA assay, whereas DNA-based assays are used in other parts of Sweden. Cytology is employed as a triage method for women who test positive for HPV. Those who test positive via self-sampling are referred for follow-up testing with clinician-collected cervical samples. Women with cytological abnormalities, defined as atypical squamous cells of undetermined significance (ASCUS) or more serious findings, are referred for colposcopic evaluation and biopsy. Current national guidelines in Sweden recommend cervical cancer screening every five years from the age of 23 to 50, and every seven years thereafter until the age of 64. Women who do not attend screening receive annual reminders, and if the final screening round is missed, these reminders continue annually until the age of 70. The National Board of Health and Welfare also recommends a risk-stratified screening approach based on HPV genotype, categorising types into high-oncogenic (16, 18, 45), mid-oncogenic (31, 33, 52, 58), and low-oncogenic (35, 39, 51, 56, 59, 66, 68) groups. Follow-up intervals are tailored according to both HPV genotype and age group. Although the Aptima mRNA assay used in southern Sweden does not enable full genotyping capacity, partial genotyping—detecting HPV 16 separately and HPV 18/45 in combination—has been available since 2021. High screening coverage is essential for the effective prevention of cervical cancer, and the nationally recommended target is set at 85%.

In 2023, the coverage rate in Region Skåne was 83.3%, compared to 77.7% at the national level among women aged 23 to 70 (105).

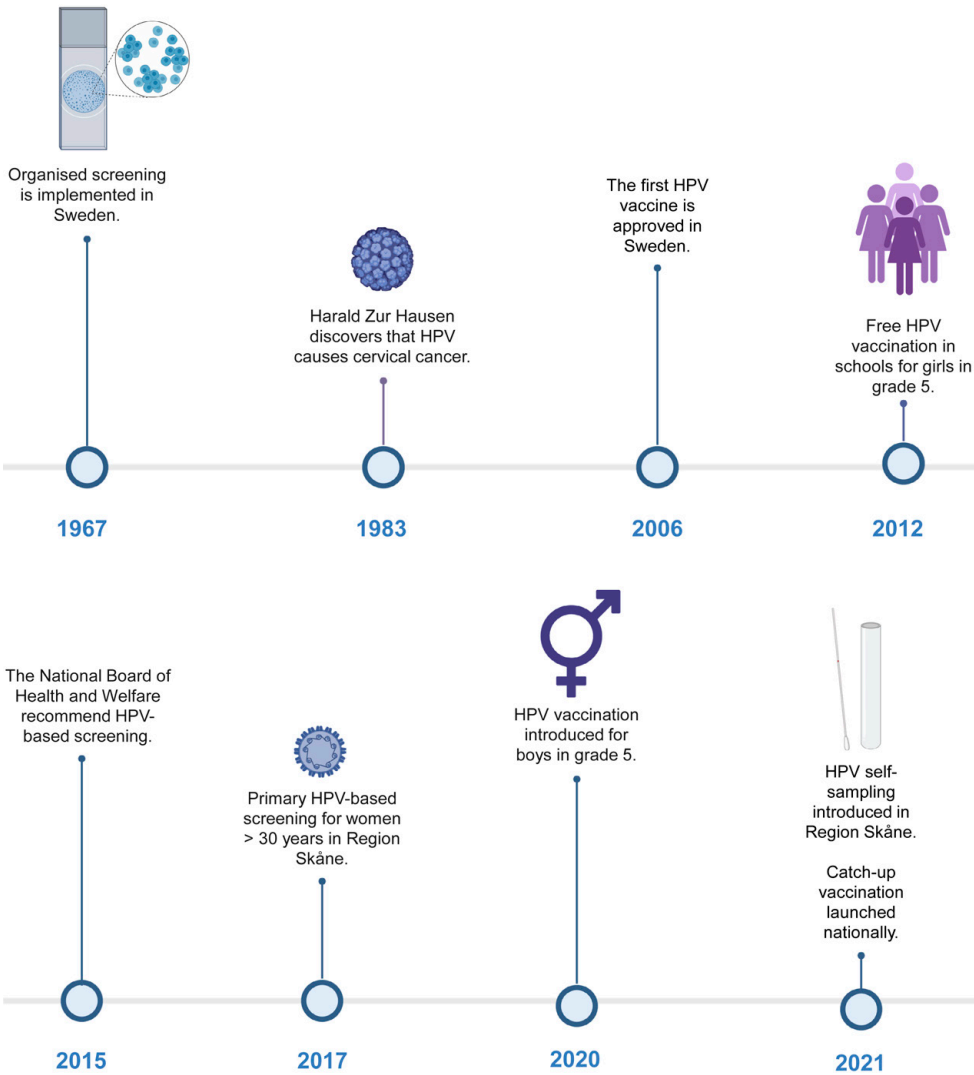

Figure 6. Milestones since the introduction of a nationally organised cervical cancer screening programme in Sweden. Created in BioRender. Hellsten, C. (2025) https://BioRender.com/vykjevl

Cytology

The widely recognised "pap smear," is named after Dr George Papanicolaou, who described the cytological characteristics associated with cervical cancer and established its application as a diagnostic tool in the 1940s (106). His method remained in use until 2009, when liquid-based cytology (LBC) replaced the conventional pap test in Sweden, enabling both HPV analysis and cytological assessment from the same sample (107). Cytology results are set according to the 2001 Bethesda Reporting system, which categorises the result as benign, Atypical Squamous Cells of Undetermined Significance (ASCUS), Low-grade Squamous Intraepithelial Lesion (LSIL), Atypical Squamous Cells, cannot exclude High-grade squamous intraepithelial lesion (ASC-H), High-grade Squamous Intraepithelial Lesion (HSIL), Atypical Glandular Cells (AGC), Adenocarcinoma *in situ* (AIS), Carcinoma *in situ* (CIS) or cancer. The former cervical squamous intraepithelial neoplasia grade 1 (CIN-1) is defined as LSIL and CIN-2 and CIN-3 are combined into HSIL (108).

Cytological assessment is subjective and can be complex; thus, maintaining high standards in the training and education of cytologists is essential. The sensitivity of cytology for the detection of CIN2+ is low, 53% compared to 96% for HPV sampling. However, the superior specificity of 5% (96% vs 91%) makes cytology a better option for triage among HPV-positive women (109).

HPV testing

A paradigm shift has emerged in cervical cancer screening, with primary HPV testing replacing traditional cytology, since HPV testing has demonstrated superior sensitivity compared to cytology testing alone (12). HPV testing offers several advantages over cytology. It is a more objective method, thus requiring less training in the interpretation of test results. Importantly, HPV testing can be performed on self-collected samples, enhancing accessibility and increasing attendance rates for the non-attenders (14-16). It also allows for the safe extension of screening intervals due to its higher sensitivity (32). Moreover, HPV testing demonstrates greater accuracy than cytology in older women, as cytology has shown reduced sensitivity in this subgroup (110).

Self-sampling

Self-samples offer multiple advantages compared to clinician-collected sampling and have demonstrated comparable reliability and diagnostic accuracy to clinician-obtained samples for HPV detection when analysed using PCR techniques (73). Self-sampling enhances accessibility and convenience, especially for women who encounter practical or emotional barriers to attending clinics (7). This is of particular

importance because the majority of cervical cancer cases occur in under-screened populations (17).

The vaginal HPV self-sampling test was introduced in southern Sweden in September 2021 for all screening-eligible women. The implementation of the self-sampling approach was driven by multiple factors, including: the enhanced sensitivity of the Aptima assay following the pre-heating step (75); findings from a randomised controlled trial in southern Sweden demonstrating equivalent detection rates of severe cervical dysplasia between self-collected vaginal and clinician-collected cervical samples (76); and the necessity for rapid adjustments to the screening programme in response to the COVID-19 pandemic (111). Nevertheless, HPV-based screening results in a higher proportion of screen-positive cases compared to cytology testing. The use of vaginal HPV self-sampling further elevates the rate of HPV detection compared to cervical sampling (112). Potential challenges associated with increased referral rates can be addressed through the implementation of clinically validated HPV assays, robust triage protocols for individuals testing positive for HPV, and extended screening intervals. Additionally, optimising the self-sampling device for improved HPV detection – while considering women's preferences regarding its design – may further enhance the overall effectiveness of the screening programme.

Despite increasing support for self-sampled vaginal HPV testing, several knowledge gaps remain compared with physician-collected cervical samples. Variability in how women perform the sampling may affect sample quality, and data on long-term outcomes and follow-up adherence after a positive viral diagnosis are limited. Optimal triage strategies for HPV-positive self-sampled women are still unclear. Although many women accept self-sampling, concerns regarding test accuracy are more pronounced for self-collected samples compared to clinician-collected samples (113). Self-sampling generally aligns well with clinician samples for hrHPV detection, although some studies report slightly lower sensitivity for identifying high-grade lesions (114). Future research should focus on optimising kit distribution, improving adherence, and evaluating long-term outcomes, including cost-effectiveness and impact on cervical cancer incidence and mortality.

Histology assessment

The classification of CIN and cervical cancer is established by histopathological assessment, which remains the gold standard for diagnosis. The Bethesda system is subsequently applied for histopathological classification as well. In CIN1, dysplastic cells are restricted to the lower third of the epithelium; in CIN2, they extend into the lower two-thirds; and in CIN3, they occupy the full epithelial thickness. If this oncogenic process progresses, it may penetrate the basement membrane, leading to the development of invasive cervical cancer (60). The two most common histological subtypes of cervical cancer are squamous cell carcinoma

(70–80%) and adenocarcinoma (20–25%). Less frequent variants include adenosquamous carcinoma, neuroendocrine tumours, and undifferentiated carcinoma (115).

Aims of the thesis

The overall aim of my thesis is the prevention of cervical cancer through the evaluation of the screening programme, with particular emphasis on the HPV self-sampling device. Below are the specific aims for Papers I-IV.

I. To investigate compliance, the prevalence of HPV, and the prevalence of severe dysplasia in participants using vaginal self-sampling, compared with participants undergoing cervical sampling performed by midwives (control arm).

II. Characterize the screening history of women diagnosed with cervical cancer to evaluate the performance of the screening programme, as well as to assess the cancer treatments given and shortcomings in the follow-up of women with cervical dysplasia.

III. Explore women's reasoning and experience when offered a self-sampling HPV test in the screening programme for cervical cancer.

IV. Evaluate the implementation and outcomes of the HPV self-sampling programme in a real-world setting.

Material and methods

Philosophy of science and overview of methods

The type of researcher you are determines how you perceive the world and interpret findings. While it is important to reflect on your own biases, assumptions and knowledge, it is equally essential to understand other researchers and their perspectives. This understanding enables you to critically evaluate studies and interpret findings (116). I have studied the accuracy of the vaginal HPV self-sampling test compared to cervical sampling, evaluated the screening history and subsequent follow-up procedures and treatments for women diagnosed with cervical cancer after the introduction of the primary HPV-based screening programme, explored experiences of women participating in the cervical cancer screening programme when offered a vaginal HPV self-sampling test, and evaluated the outcomes of the primary HPV self-sampling programme in southern Sweden within a real-world setting. Papers I, II, and IV are quantitative studies performed according to the post-positivism paradigm, whereas Paper III is a qualitative study according to the interpretivism paradigm (see Figure 7).

The four major paradigms in medical research are positivism, post-positivism, critical theory, and interpretivism. The research question determines the most appropriate paradigm. A paradigm is a philosophical framework that incorporates ontology and epistemology, shaping and influencing the research process and choice of methodological approach. Ontology refers to the nature of reality, whereas epistemology concerns the nature of knowledge and how it is acquired. Each paradigm differs in its ontological and epistemological stance, and researchers must critically reflect on the assumptions they adopt in their specific research project, as these assumptions guide the choice of methodological tools and influence the interpretation of findings (116-118). A positivist researcher employs a quantitative methodology and emphasises objectivism, numerical data, and generalisability. In contrast, an interpretivist researcher adopts a qualitative methodology, relying on descriptive data (e.g., interviews, open-ended questionnaires, focus groups), subjectivism, and research conducted within a specific context. Rather than aiming for generalisability, interpretivist research seeks to obtain a deeper understanding of complex real-world challenges and to explore experiences, behaviours and perceptions. To ensure the credibility and coherence of the research process, there must be internal consistency within the chosen paradigm (see Figure 7) (117, 118).

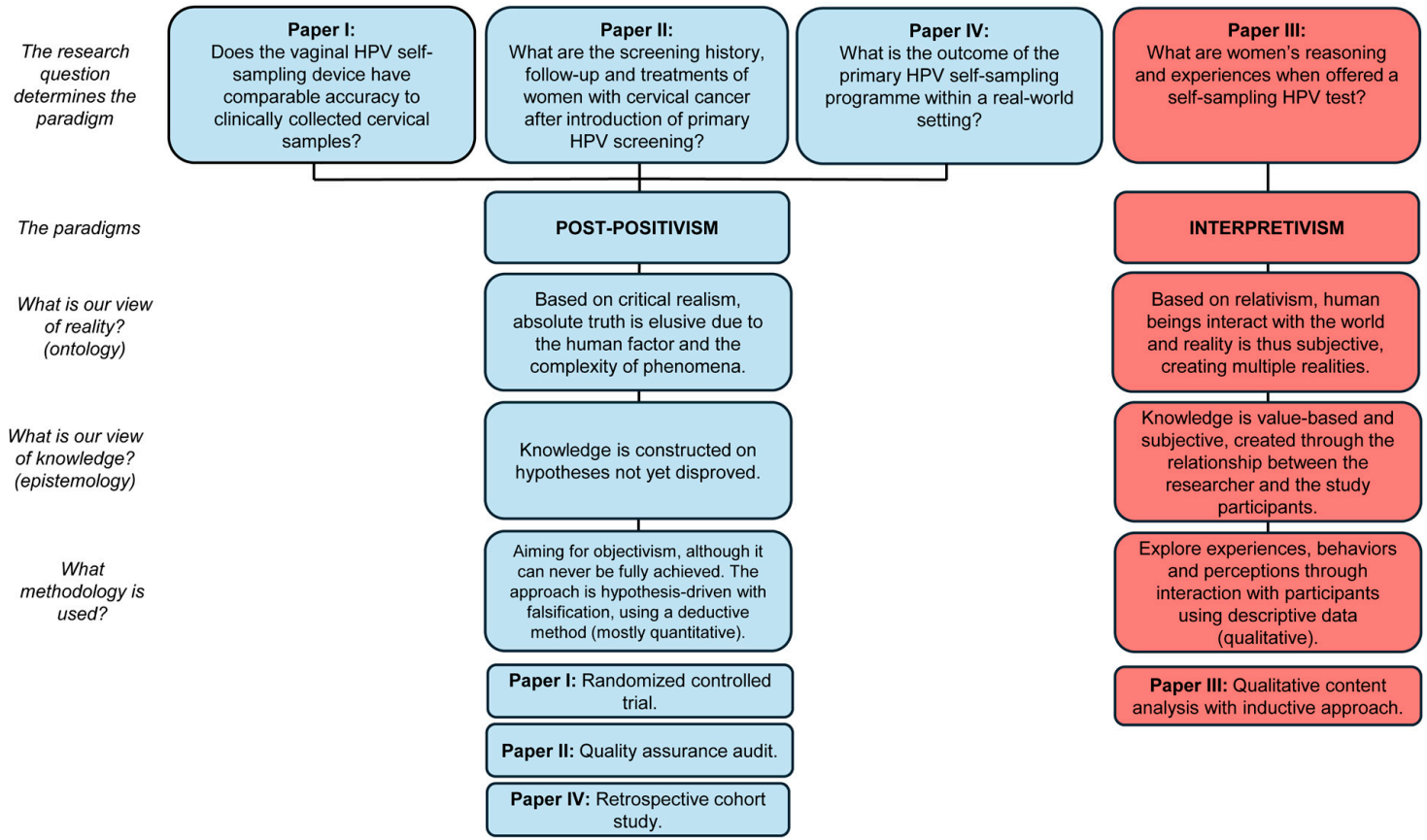

Figure 7. Two paradigms of medical research in relation to my studies (Papers I-IV) (116-118)

Paper I

Study design

Paper I is a randomised controlled trial conducted to evaluate the effectiveness of HPV self-sampling compared to routine clinician-collected cervical samples within the framework of the organised cervical cancer screening programme in southern Sweden.

Study population

The study targeted women aged 30–64 years who were scheduled to receive a routine cervical cancer screening invitation during the autumn of 2019. Women aged 40–42 were excluded, as they were, at the time of the study, invited for co-testing with both HPV and cytology. In total, 29,604 eligible women were included. Using the FlexlabInvitation software (Tieto Inc., Stockholm, Sweden), participants were randomly allocated in a 1:1 ratio into two age-matched groups: the intervention group (self-sampling) and the control group (clinician-collected sampling). The large sample size in both study arms reflects the scalability of the approach within the general Swedish population.

Intervention group

A total of 14,765 women were assigned to the self-sampling group. HPV self-sampling kits (Multitest Swab Specimen Collection kit, Hologic) were mailed to the participants on September 20 and 26, 2019. Each kit included written and illustrated instructions, informed consent documents, and all necessary equipment for self-collection. Samples were returned by prepaid mail to the Department of Pathology at Lund University Hospital. All returned samples received by January 31, 2020, were included in the analysis. No reminders were issued to non-responders, who would instead be re-invited for routine screening in the following year.

Control group

The control group consisted of 14,839 women who received standard invitations for primary HPV screening between August 27 and October 18, 2019. These women underwent cervical sampling performed by midwives according to regional screening protocols. This time period was selected to match the recruitment volume of the intervention group, given that approximately 9,400 women are invited for screening each month in the region.

HPV analysis

Cervical samples were collected in 20 mL of PreservCyt solution, while self-collected vaginal samples were preserved in 2.9 mL of Multitest Specimen Transport Media. A pre-heating step at 90°C for 75 minutes was applied exclusively to self-collected samples to enhance assay sensitivity. This protocol was introduced in the southern region of Sweden on January 17, 2019. HPV testing was performed using the Aptima HPV mRNA assay (Hologic) on a Panther instrument for both the vaginal and cervical samples. Invalid results were retested in duplicate and counted as valid if at least one test yielded a valid result. Women with invalid results were excluded and received an invitation for cervical sampling by midwives instead.

In retrospect, an evaluation of the Aptima assay's diagnostic performance through direct comparison of self-collected and clinician-collected samples could have added further value to this study.

Data sources and management of follow-up procedures

Data on diagnostic outcomes were retrieved from the regional Laboratory Information Management System (LIMS) and the electronic health record system Melior (Cerner Inc., Stockholm). Women with HPV-positive vaginal self-samples were invited to attend follow-up appointments with midwives within three months for clinician-collected cervical samples. These samples underwent co-testing with cytology and repeat HPV analysis. In the intervention arm, cytological assessment was performed only for women who tested positive for HPV. Cytological assessments were conducted by trained cytotechnologists at the Department of Clinical Pathology, Lund University Hospital. In both the intervention and control arms, cytotechnologists were aware of the participants' HPV-positive status at the time of cytology interpretation. If indicated, women were referred for further gynaecological evaluation, including colposcopic examination and cervical biopsy. Histological specimens were submitted to the Department of Pathology in Lund for microscopic examination and diagnostic interpretation.

Outcome measures

The primary outcome was the proportion of histologically confirmed HSIL, AIS, or cervical cancer. Secondary outcomes included participation rates, HPV positivity rates, and prevalence of abnormal cytology.

Statistical analysis

Statistical comparisons were based on binomial distribution. Exact CI intervals were reported, and Pearson's chi-square test was used to compare HSIL and AIS rates between groups. A significance level of $p < 0.05$ (two-sided) was used.

Power calculations assumed an HSIL+ prevalence of 1.6% among screened women in Sweden. For non-inferiority testing, a minimum of 5,393 women (2,697 per group) was needed to achieve 90% power with a one-sided 95% CI, excluding a >1% difference in favour of the control group. A second power analysis to detect a 1% absolute difference with 90% power and alpha = 0.05, required at least 2,284 women per group.

Paper II

Study design and setting

This study was conducted as a retrospective quality assurance audit to evaluate the performance of the primary-based HPV cervical cancer screening programme by examining the screening histories, treatment pathways, and follow-up adequacy among women diagnosed with cervical cancer. The study was carried out in Region Skåne, Sweden, and covered all cancer cases detected between January 1, 2017, and December 31, 2020.

Study population

Women diagnosed with invasive cervical cancer during the defined study period and residing in Region Skåne were included. A total of 91 cases were excluded for the following reasons: non-invasive cancer (n = 13), recurrent cervical cancer (n = 2), primary tumour site other than the cervix (n = 40), residence outside Region Skåne (n = 24), diagnosis outside the specified time frame (n = 9), duplicate entries (n = 2), and incorrect personal identification numbers (n = 1).

Data sources and analysis of data

Data were collected from the National Cervical Cancer Prevention Registry, the Region Skåne Labmedicin database, and the electronic health record system Melior (Cerner Inc., Stockholm, Sweden). HPV testing was primarily performed on LBC samples using the Aptima HPV mRNA assay (Hologic Inc.), which detects 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 82). All HPV-negative samples were re-rested, and in cases where LBC samples were unavailable (n = 28), histopathological formalin-fixed paraffin-embedded (FFPE) tissue

specimens were also subjected to broad-range HPV typing using Luminex analysis. The date of diagnosis was defined as the date when the pathologists responded to a biopsy they assessed as invasive cancer.

Data categorisation

The following variables were collected for each patient: personal identification number, age at diagnosis, date of diagnosis, stage of disease according to the International Federation of Gynaecology and Obstetrics (FIGO) classification, mode of cancer detection (screening, symptoms, or incidental finding), histopathological subtype, pregnancy status, presence of stump cancer, screening history, prior cervical dysplasia, treatment history, and HPV testing results.

All cases were staged according to the 2009 FIGO classification system to ensure consistency throughout the study period, as the revised FIGO system was introduced in 2019.

Women were categorised into one of four groups based on their screening history:

- **Regularly screened:** Women with documented participation in at least two consecutive screening rounds prior to diagnosis.
- **Irregularly screened**: Women who had missed at least one of the two most recent screening rounds.
- **Interval cancer**: Women who developed cervical cancer within the interval between two screenings, i.e. symptomatic cancer diagnosed within 3.5 or 5.5 years after the last screening. In accordance with definitions from the National Cervical Screening Registry (NKCx), screening coverage is defined as participation within a 3.5- or 5.5-year interval, which also served as the cut-off point for this study. Notably, if a woman had only two consecutive screening tests in her lifetime, she was classified as absent from screening, except in cases where these were her first two eligible screening rounds.
- **Above screening age**: In 2017, the final screening round was performed at the age of 65, but this was subsequently changed to the age of 64 from 2018 onward. Above screening age was defined as above the age of 65 if diagnosed in 2017, and above the age of 70 if diagnosed between 2018 and 2020 since annual reminders were sent to women who did not attend their final screening round at age 64 until the age of 70.

Further sub-categorisation was carried out to capture cases of inadequate diagnostics or follow-up, based on current regional screening guidelines. This included women with a prior history of cervical dysplasia who either declined follow-up or were not referred for colposcopy or further investigation. Importantly,

a prior history of cervical dysplasia was defined as dysplasia occurring earlier in life, and not related to the most recent screening test that led directly to cancer detection.

Additionally, women classified as being above screening age were further stratified based on lifetime screening participation: those who had never been screened versus those who had been previously screened, and whether the cancer was detected within or beyond five years from their last screening episode.

Finally, it is worth noting that additional variables, such as a history of sexually transmitted infections, sociodemographic factors, or HPV genotype persistence, could have provided valuable insights, particularly in assessing whether HPV-positive cervical tumours were a result of a new acquisition or recurrent HPV infection. However, such data were not available within the scope of this study.

Statistical analysis

Statistical comparisons were based on the binomial distribution, and exact confidence intervals are given. Pearson's chi-square test and Fisher's exact tests were used for comparisons. All tests were two-sided and P-values <0.05 were considered statistically significant. IBM SPSS Statistics Version 28 was used for calculations.

Paper III

Study design

When conducting qualitative research, the objective is to obtain a deeper understanding of complex real-world challenges and explore experiences, behaviours and perceptions through the analysis of descriptive data (119). There are various qualitative study designs, with ethnography, phenomenology, grounded theory, and content analysis being some of the most used approaches (120). The research question determines the appropriate study design. In this qualitative study, we aimed to explore women's reasoning and experience when offered a self-sampling HPV test in the routine screening programme. Therefore, content analysis was the most appropriate study design, since the data is based on narratives from questionnaires (121, 122). The methodological approach is inductive, as the study aims to generate new insights into the acceptability of the self-sampling device and explore the reasons for non-attendance. This contrasts with the deductive approach, which focuses on testing existing theories (123), and the abductive approach, which combines the inductive and deductive approaches (124).

Sampling strategy and study setting

The recruitment strategy in this study was purposive sampling, a method in which researchers deliberately select participants who are most likely to provide relevant and insightful information to address the study's objectives, i.e. key informants (125). Naturally, the most appropriate study population consisted of women invited to participate in the cervical cancer screening programme following the implementation of the HPV self-sampling device in September 2021. To achieve a variation and spread of data collection from key informants, i.e. maximum variation sampling, three different groups of women were recruited based on their screening history (125). Both adherent attenders, non-attenders (absent for at least two screening rounds), as well as a certain number of women with cervical dysplasia were recruited. The computer program Flexlabinvitation (Tieto Inc., Sweden) was used to find the attenders and non-attenders of the screening programme based on the inclusion criteria. Women aged 23-29, 30-49, and 50-70 were then randomly selected to achieve equal representation across age groups. All women with cervical dysplasia and subsequent conisation during 2021 and 2022 after a positive HPV self-sampling test were recruited from the LIMS database.

In qualitative research, it is not possible to predefine an appropriate sample size, since the concept of "data saturation" is employed, referring to the point at which data collection continues until no new evaluative information emerges. The sample size in qualitative research is typically smaller than that in quantitative studies. This is because qualitative research seeks to achieve a deeper understanding of a phenomenon rather than to generalise findings to the broader population. However, the diversity of experiences and the richness of information provided by participants selected as key informants allow for a smaller study sample (125). Several factors determine a sufficient sample size. First, the scope of the research question affects the number of participants required. A broader research question necessitates a larger sample size, whereas a narrower question may require fewer participants. In this study, the research question is relatively broad; therefore, a larger sample size is needed. Second, the target population's knowledge of the phenomena under study is an important consideration. The eligible participants in this study were women due to undergo cervical cancer screening and had received a self-sample device. To enrich the data, we aimed to explore perspectives from participants with different screening histories and ages. By employing a purposive sampling strategy, we sought to increase specificity, thereby reducing the need for a greater sample size. Third, existing knowledge on the topic also impacts sample size. When no prior theoretical framework exists, a larger sample is typically needed. In this study, we adopted an inductive approach to gain new insights into the acceptability of self-sampling following its introduction as a primary screening method. Although previous studies have examined women's acceptability of HPV self-sampling and their responses to HPV-positive results and cervical dysplasia, these were conducted before HPV self-sampling was implemented as the primary screening method.

Ultimately, the data collection strategy also influences sample size. In this study, data were collected through a questionnaire with open-ended questions. Since the questionnaire was performed without any interaction between the researcher and participant, no follow-up questions could be asked. If in-depth interviews had been conducted instead, a smaller sample size would have been sufficient, as interviews provide more detailed and richer data compared to a questionnaire (126).

We anticipated that the response rate among non-attenders would be low, particularly given that half of the women invited had not returned their self-sample. Therefore, we sent an invitation letter for participation to 1,500 non-attenders (750 had collected and returned a self-sample, and 750 had not). In total, there were 115 women with a conisation after a positive HPV self-sample, and therefore, we chose to include all these women. For the regular attenders at the screening programme, we decided on 300 participants and estimated that less than 50% might answer the questionnaire. It is difficult to predict in advance the number of participants needed to achieve data saturation and at the same time avoid excessive redundancy, which could hinder effective analysis, in the descriptive text. We therefore planned to conduct follow-up semi-structured interviews if the data was not sufficient for analysis. However, we reached an agreement in our research team on data saturation after analysing the 173 questionnaires up until September 2023, since no new observations emerged in the data. Nevertheless, we did conduct a follow-up study with non-attenders who had submitted the questionnaire, aiming to explore their reasons for non-attendance through semi-structured in-depth interviews. While this data is not included in the thesis, data collection has been completed, and a manuscript is currently being prepared.

Development and translation of the questionnaire

The COREQ (Consolidated Criteria for Reporting Qualitative Research) checklist, consists of 32 criteria for reporting qualitative research, and was used in this study (127). Questions were designed to be open-ended and neutral, allowing women to freely express their thoughts and feelings. Additionally, background questions addressing risk factors for an HPV infection were included (See supporting information, Appendix 1). A pilot study was performed in June 2022 on three women who had an appointment at the colposcopy unit at Lund University Hospital, and on four women without cervical dysplasia. Minor amendments to the open-ended questions were then made. The first two questions merged into only one question, and one question was removed since it did not add more valuable information.

The original Swedish questionnaire was translated into English by four independent bilingual translators, all native Swedish speakers with high proficiency in English. Among them, two were physicians and one had expertise in qualitative methodology. After a collaborative review and discussion within the research team,

CH selected the version that most accurately captured the intent and content of the original. To ensure fidelity, the chosen English version was then back-translated into Swedish by two separate translators. The research team carefully evaluated the back-translation, and final agreement was reached on both language versions of the questionnaire.

Data collection and analysis

In content analysis with an inductive approach, the data is initially divided into meaning units, which consist of words, sentences, or phrases that convey a shared central meaning. These meaning units can be condensed at a descriptive level without altering their original meaning. Subsequently, codes are generated by assigning a word or a sentence to each meaning unit to capture its essence. The purpose of coding is to facilitate the organisation and interpretation of the data when later forming categories. In each category, the content shares a common characteristic, and data cannot be included in more than one category, nor omitted entirely. The categories describe the text on a manifest level (the objective meaning of the text). To describe the content on a latent level (the underlying meaning), a theme is created from the emerging categories (121). Meaning units, codes and categories were first outlined by CH, with further revisions by and discussion with LM and CB. Ultimately, the emerging categories were analysed and integrated into one overarching theme to illuminate the latent content.

To ensure trustworthiness, measures of qualitative criteria in terms of credibility, transferability, dependability, confirmability and reflexivity were discussed and applied throughout the research procedure (128). These measures are referred to as internal validity, generalisability, reliability and objectivity in quantitative research. Strategies to increase credibility were maximum variation purposive sampling (125) and investigator triangulation (128). Maximum variation purposive sampling means that researchers deliberately select participants who are most likely to provide relevant and insightful information and who have diverse experiences related to the aim of the study (125). Investigator triangulation means that several researchers analyse the data (128). LM has expertise in qualitative research and public health (associate professor, PhD); CH (PhD student, resident) and CB (professor, MD, PhD) are clinicians with knowledge of the clinical aspects and experience of interacting with women at colposcopy clinics. Transferability (to what extent our results can be applied in other contexts and with other participants) (128, 129) was ensured through a description of the study's context and by presenting the findings in a structured manner with quotations corresponding to the core of each emergent category from the data analysis. Dependability refers to the stability of our findings over time during the analysis process (128, 129). This was improved by consistent documentation of data, and considerations, reflections and decisions that evolved during the research process were shared as an audit trail. Confirmability (findings

are explicitly grounded in the data and can be independently verified by other researchers) and reflexibility (critical self-reflection when analysing data) (128) were strengthened by the members of the research team presented above, who contributed to the data analysis with diverse perspectives. No prior relationship existed between the researchers and study participants during the conduct of this study. Continuous field notes on decisions, reflections and interpretations during the data analysis were shared and discussed within the research team. Subsequently, the quality of the research has been increased by bringing different perspectives to the data analysis, which minimises our own biases, assumptions or interpretations concerning the descriptive text.

Paper IV

Study design

Paper IV is a population-based study that evaluates the performance of the HPV self-sampling programme implemented in Region Skåne, Sweden within a real-time setting. The analysis covered the period from September 1, 2021, to December 31, 2024. To allow for complete follow-up of participants with positive self-sampling results at the end of the study period, an additional four-month extension was applied to capture follow-up samples and outcomes.

Data Sources

Data collection was conducted through two primary systems: the Laboratory Information Management System (LIMS) database and Flexlabinvitation software (Tieto Inc., Stockholm, Sweden), which is used to manage invitations and data flow within the regional screening programme. The Melior medical record system was used to collect additional data on FIGO stage and screening history for the identified cervical cancer cases.

Self-sampling procedure and laboratory analysis

Self-sampling kits were mailed to eligible women at their home addresses. Each kit contained a Multitest Swab Transport Media (STM) tube with 2.9 mL of transport fluid and an instruction leaflet with both written and illustrated guidance on how to perform the vaginal self-sampling. Participants returned complete samples to the Department of Pathology, Lund University Hospital. Upon receipt, the vaginal self-samples underwent a pre-heating step at 90°C for 1 hour and 15 minutes, to enhance assay sensitivity. After cooling to room temperature, the samples were

analysed using the Aptima HPV mRNA assay (Hologic Gen-Probe, San Diego, CA, USA).

Follow-up procedures

Women who tested positive for high-risk HPV were invited to midwife-led outpatient clinics. Follow-up samples were included if collected through the end of the fourth subsequent calendar month from the vaginal self-sample. At these follow-up visits, a cervical sample for LBC and repeat HPV testing were collected. Extended genotyping with separate detection of HPV16 and combined detection of HPV18/45 was introduced on the 1st of February 2021. Cytological results were classified according to the Bethesda system. Women with findings of ASCUS or worse were referred for further evaluation at colposcopy clinics, where biopsies and additional cytological and HPV assessments were performed. All laboratory analyses, including HPV testing, cytology, and histopathology, were conducted at the Department of Pathology, Lund University Hospital.

Outcome measures

The primary outcome of the study was the number of histopathologically confirmed cervical dysplasia cases through the end of the 12th subsequent calendar month from cervical sampling, including HSIL, ASC-H, AIS, CIS, or invasive cervical cancer.

Secondary outcomes included:

- Adherence to self-sampling kit return
- Adherence to follow-up cervical sampling among HPV-positive women through the end of the fourth subsequent calendar month
- Number of HPV-positive results in both vaginal and cervical samples
- Number of cytological abnormalities

Statistical analysis

Statistical analyses were performed using binomial distribution-based comparisons and exact CI. A two-tailed P-value of < 0.05 was used to determine statistical significance. IBM SPSS Statistics Version 28 was used for calculations.

Ethical considerations

We obtained approval from the Regional Ethics Board in Lund (DNR 2013-390 with amendment 2018-466) for studies I, II and IV. For study II, we also received approval to access the local journal system Melior and the Laborit database from the Health and Welfare committee at Region Skåne DNR 041-19. We obtained approval from the Regional Ethics Board in Lund for study III (DNR 2022-03618-01) and approval for disclosure of patient data (Region Skåne KVB 300-22).

In line with the fundamental ethical principles first articulated by Hippocrates over 2,500 years ago: "never harm, cure if possible, often relieve, always comfort", medical research should always be designed to minimise risk and maximise potential benefit to participants and society. It is the researcher's responsibility to conduct medical studies on humans according to the ethical principles of the Declaration of Helsinki. The primary focus must always be the well-being of the participants, with full respect for their rights, autonomy, and the confidentiality of their personal information. A thorough risk–benefit assessment should be conducted prior to initiating the research, and ongoing evaluation is essential to ensure participant safety, study effectiveness, and adherence to high-quality standards (130).

In Paper I, self-sampling kits were not part of the recommended screening protocol at the time. However, in studies II–IV, the standard care provided to women within the screening programme was maintained without modification. No research-related procedures were introduced that could have negatively impacted the physical or mental health of the participants. The participants were required to provide written informed consent in order to take part in the study in Paper I. All data were treated confidentially and anonymised during statistical analysis. Women who did not return their self-sampling kits received a standard invitation to participate in the routine screening programme, and appropriate clinical follow-up was arranged for women who tested positive on their self-sample. In papers II and IV, data were obtained from quality registries. These data were handled in a confidential manner and presented anonymously. All women in Sweden are informed of their right to opt out of inclusion in quality registries both when receiving a screening invitation and when undergoing treatment. Lastly, in Paper III, the participants received a letter containing information about the study objectives, and written informed consent was required to complete the questionnaire. All returned questionnaires were pseudonymised to ensure participant confidentiality.

In all four studies, HPV testing was performed using the Aptima mRNA assay, which provides a higher HPV prevalence compared to other DNA-based assays for self-samples when the pre-heating step is used, as in Papers I, III, and IV. While previous studies have reported negative emotional responses following a positive HPV result (131-133), no long-term anxiety has been observed in two other studies (134, 135). Given that follow-up appointments for women testing HPV-positive via vaginal self-samples are typically scheduled within three months, long-term adverse psychological outcomes solely due to a positive vaginal HPV result are not anticipated. During the implementation of the self-sample device in 2021, most enquiries from women participating in the screening programme in the southern part of Sweden related to concerns about the sampling process itself. However, by 2022, the most common reason for contacting the coordinator was related to testing HPV-positive, with individuals wanting a prompt follow-up appointment with a midwife. This might highlight the importance of giving sufficient information about HPV and the rationale behind the screening protocol. As HPV-based screening leads to a higher number of follow-up procedures than conventional cytology-based screening, the use of effective triage methods is essential. In the current cervical cancer screening programme in southern Sweden, extended HPV genotyping, which distinguishes HPV type 16 and combines detection of 18/45, is used for risk stratification to determine the appropriate surveillance interval. Cytology remains the standard triage test for HPV-positive women. However, neither cytology nor HPV genotyping provides prognostic information regarding infection persistence. For future screening programmes it is also important to reflect upon the HPV genotypes detected by the used assays. Is it worth finding all very rare HPV types in screening programmes, or should the screening programme concentrate on finding the most common carcinogenic HPV types to avoid overtreatment? To mitigate the ethical trade-off between maximising cervical cancer prevention and minimising overtreatment-related complications, more precise risk-stratification strategies for HPV-positive women are warranted that enhance the effectiveness and precision of the screening programme. The current Swedish national guidelines recommend classification and triage based on categorisation of HPV types as high-oncogenic (16, 18, 45), mid-oncogenic (31, 33, 52, 58), or low-oncogenic (35, 39, 51, 56, 59, 68) (44).

A potential problem with mailed self-samples is the risk of tests being lost in the mailing process; however, given that women who did not respond to the vaginal self-sampling received an invitation to the conventional screening programme, no adverse outcome can be identified. Data concerns with a self-sample include determining who actually took the test. Perhaps it was given to someone else in the household or it was sent to the wrong home address. Therefore, to minimise these potential inaccuracies, each woman is asked to double-check her own social security number that was sent with the self-sampling kit and then label the test tube accordingly. Women sending in a sample with another woman's ID will not be able

to be ruled out, but those who test positive for HPV will have a second cervical HPV sample at follow-up.

The results from Paper III indicated that HPV self-sampling may help reduce both emotional and practical barriers to participation in cervical cancer screening. However, some participants reported anxiety related to cervical dysplasia or following a positive HPV result, highlighting the importance of healthcare professionals providing personalised information and emotional support from healthcare professionals to mitigate negative emotional responses. When interacting with these patients, it is therefore essential for healthcare providers to be mindful of the psychological impact of test results and to offer clear, compassionate guidance throughout the follow-up process. Additionally, three participants invited to answer the questionnaire contacted the researcher (Caroline Hellsten) via email or text message to request a self-sampling kit, stating that they had not received one. In response, the responsible cytodiagnostician was contacted to verify the status of kit distribution. Furthermore, one woman who had completed the questionnaire and participated in a follow-up interview for an upcoming manuscript reached out to the researcher a few months later with questions regarding her own test results and requested guidance. Her concerns were addressed accordingly.

Women who completed the questionnaire did so independently in their own environment, without any direct interaction with the researcher. The questionnaires were submitted anonymously and had no influence on the care provided within the cervical cancer screening programme. Among women who expressed a preference for clinician-based cervical sampling, several emphasised the value of personal contact and the opportunity to receive a general health check-up, during which they could raise additional concerns regarding their reproductive health. It is important to note that the primary aim of the cervical cancer screening programme is to prevent cervical cancer, not to serve as a general gynaecological consultation. However, women who test positive for HPV via self-sampling are referred for a follow-up appointment with a midwife for clinician-collected cervical sampling. In this study, six women chose not to perform the self-sampling test. For these individuals, as well as for certain subgroups—such as women with rheumatic disease, obesity, tremors, or other conditions that may hinder the self-sampling process—clinician-based cervical sampling may be more appropriate. It should be emphasised that women always have the option to choose clinician-based cervical sampling instead of self-sampling within the national screening programme.

To conclude, all the papers included in this thesis aimed to evaluate the effectiveness of the cervical cancer screening programme and to provide deeper insights into the performance of primary HPV-based screening. Identifying targeted improvements to the screening programme is essential to support the long-term goal of eliminating cervical cancer. Furthermore, exploring women's experiences within the screening programme is critical, as they have important implications for clinical practice and

programme implementation. Women of screening age stand to benefit from enhancements to the cervical cancer screening program.

Results

Paper I

Main findings

The vaginal self-sampling approach detected a similar proportion of severe cervical dysplasia as regular midwife-collected cervical screening, 0.48% (95% CI, 0.3–0.72%) in the self-sampling group and 0.47% (95% CI, 0.3–0.66%) in the cervical sampling group, respectively.

The participation rate was 33.5% in the self-sampling arm and 47.5% in the control arm ($p < 0.0001$). HPV was detected in 17.1% (95% CI, 16.1–18.23%) in the self-sampling arm and 4.5% (95% CI, 4.0–5.0%) in the cervical sampling arm.

Among the vaginal HPV-positive women, 91.2% (743/815) attended follow-up for cervical sampling by midwives. Thirteen women were excluded from the follow-up due to a previous total hysterectomy. At follow-up, 37.3% (272/730) of the women were HPV-positive in the cervical sample.

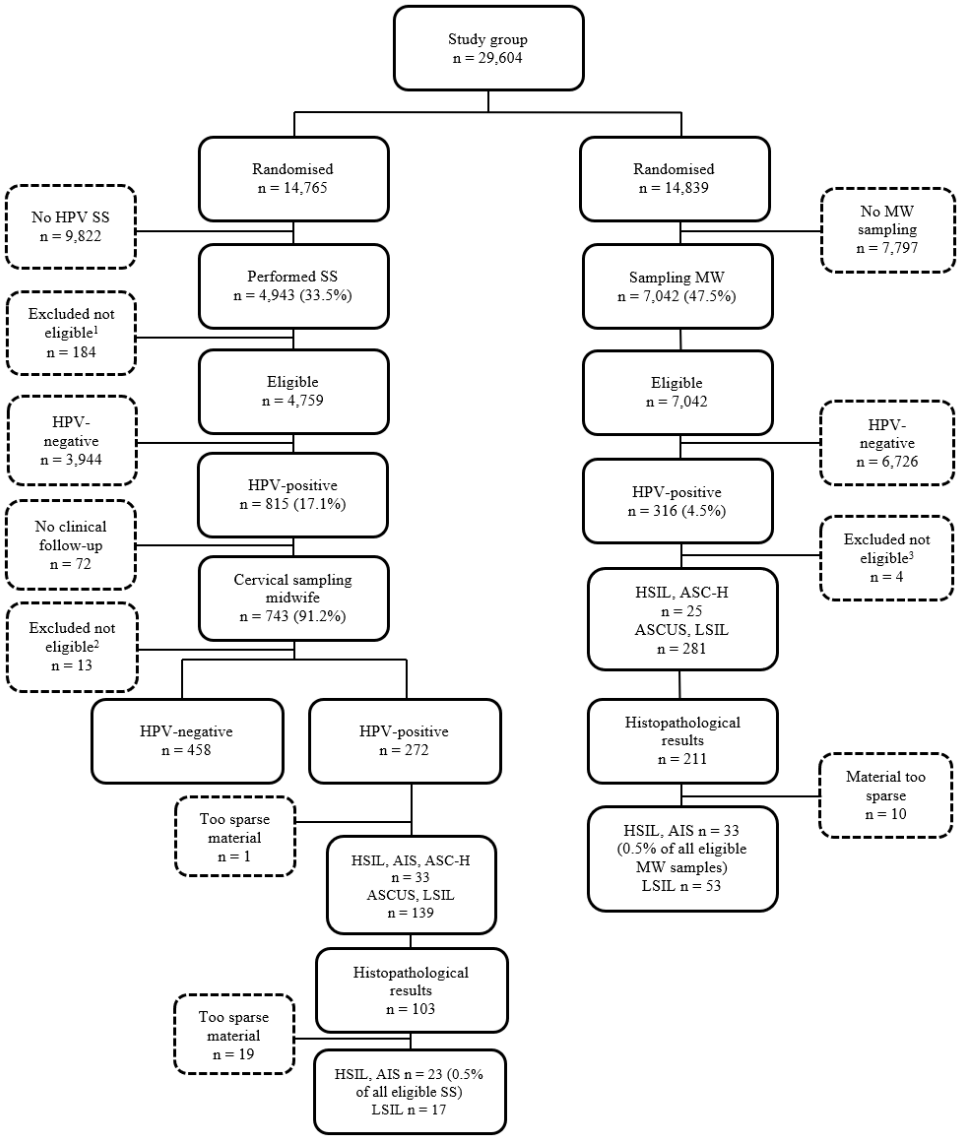

Figure 8. Study design. AIS, adenocarcinoma in situ; ASC-H, atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion; ASCUS, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HSIL, high-grade intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; MW, midwife; SS, self-sampling. [1]Not eligible due to invalid HPV analysis (n =184, 3.7%). [2]Not eligible due to previous total hysterectomy (n =13). [3]Not eligible due to previous total hysterectomy (n=2) or due to material being too sparse for cytology analysis (n =2).

Cytology

Self-sampling arm

Using cytology, HSIL was diagnosed in 7.4% of women (20/272), ASC-H in 4.4% (12/272) and AIS in 0.4% (1/272) (Table 1). At follow-up, the positive predictive value (PPV) was 12.1% (33/272) for the HPV test to detect any of those cytological diagnoses.

Table 1. Cytology and HPV results at follow-up with midwife in self-sample HPV-positive women.

Cytology	HPV-positive (n)	HPV-negative (n)	Total (n)
Benign	98	432	530
ASCUS	88	16	104
LSIL	51	3	54
HSIL	20	0	20
ASC-H	12	0	12
AIS	1	0	1
Atypical cell of undetermined/unclear cell type	1	0	1
Glandular cells with atypia	0	0	0
Atypical squamous epithelium	0	1	1
Not eligible*	1	6	7
Total	**272**	**458**	**730**

* Not eligible due to material being too sparse for cytology analysis.

Control arm

HSIL in cytology was diagnosed in 4.5% of women (14/316) and ASC-H in 3.5% (11/316) (Table 2). At follow-up, the PPV was 7.9% (25/316) for the HPV test to detect any of those cytological diagnoses.

Table 2. Cytology results in HPV positive women with primary cervical sampling (control arm).

Cytology	n	%
Benign	5	1.6
ASCUS	190	60.5
LSIL	91	29.0
HSIL	14	4.5
ASC-H	11	3.5
AIS	0	0.0
Atypical cell of undetermined/unclear cell type	0	0.0
Glandular cells with atypia	1	0.3
Atypical squamous epithelium	0	0.0
Not eligible*	2	0.6
Total	**314**	**100**

*Not eligible due to material being too sparse for cytology analysis.

Histology

Self-sampling arm

Histopathology was performed on 103 women, and HSIL was diagnosed in 21.4% (22/103) and AIS in 1.0% (1/103) (Figure 8). The PPV was 22.2% (23/103) for the HPV test to detect any of those histological diagnoses.

Control arm

Histopathology was performed on 211 women and HSIL was diagnosed in 13.7% (29/211) and AIS in 1.9% (4/211) (Figure 1). The PPV was 15.6% (33/211) for the HPV test to detect any of those histological diagnoses.

Paper II

Main findings

In total, 247 patients were diagnosed with invasive cervical cancer in Region Skåne, Sweden, between 2017 and 2020. The most common screening history in women with cervical cancer was irregular screening (143, 57.9%), followed by women being above screening age (44, 17.8%). HPV was expressed in 96% of the cervical tumours. The screening programme detected the disease in 96 (38.9%) of the patients, while 149 (60.3%) were diagnosed through symptoms and two (0.80%) because of incidental findings.

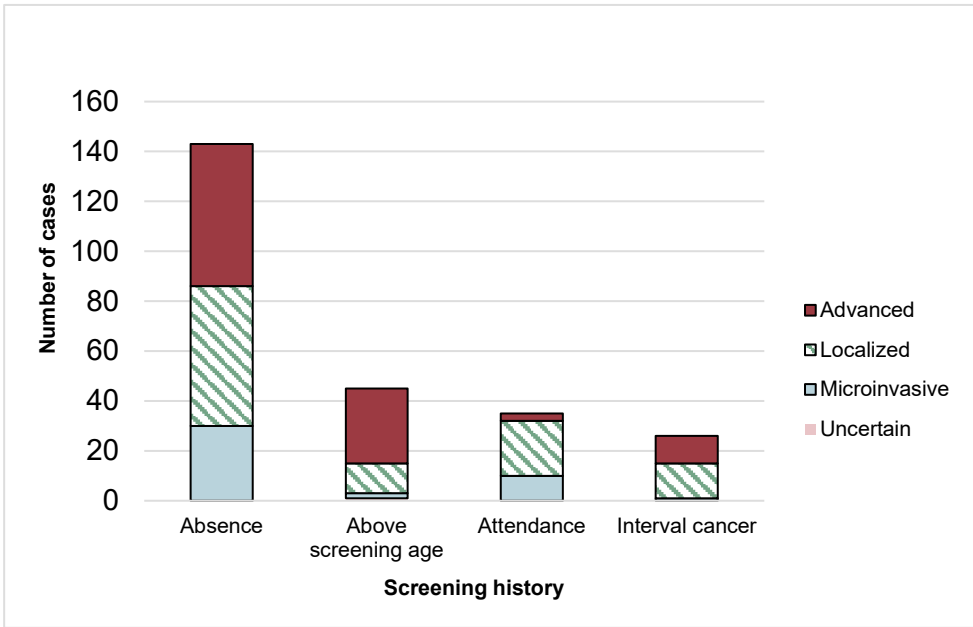

Figure 9. Screening history in women with cervical cancer in relation to the spread of cervical cancer at diagnosis.

Table 3. Screening history.

	No. of cases (n)
Attendance	**35**
Normal screening history	14
Abnormal screening history with adequate follow-up according to the screening program	
High-grade cervical dysplasia	16
Low-grade cervical dysplasia	5
Above screening age	**44**
Normal screening history	33
Abnormal screening history	
High-grade cervical dysplasia	
Inadequate or declined follow-up	2
Adequate follow-up	4
Low-grade cervical dysplasia	
Adequate follow-up	5
Irregular screening history	31
Diagnosed > 5 years since last screening	7
Diagnosed < 5 years since last screening	4
Absence	**143**
Unscreened in the most recent round of screening	29
Screened in the most recent round but unscreened in the previous round	27
Unscreened in the past two screening rounds	87
Interval cancer	**25**
Normal screening history	
Irregular screening history	3
Regular screening history	18
Abnormal screening history	
High-grade cervical dysplasia	
Inadequate or declined follow-up after AIS	1
Adequate follow-up after HSIL	1
Low-grade cervical dysplasia	
Adequate follow-up	2

Age groups in relation to tumour size and spread of cervical cancer

Microinvasive and localised cervical cancers were more common in younger age groups and decreased with age (Figure 10; $P < 0.001$). The highest incidence rate of cervical cancer was in women aged 40– 49. The highest incidence of early stages of cervical cancer was seen for women aged 30–49. Among women 23–49 years of age, 26% (33/126) were diagnosed with advanced stages, in contrast to women 50 years of age or above, where 55% (67/121) presented with advanced stages of cervical cancer ($P < 0.001$).

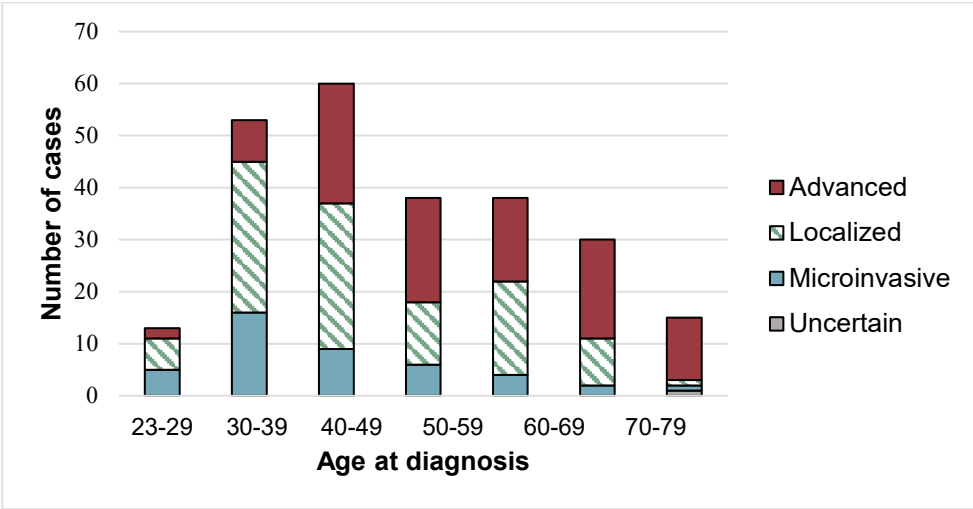

Figure 10. Age groups correlated to the size and spread of cervical cancer at diagnosis.

FIGO stage in relation to mode of detection

Attendance at the screening programme resulted in detection of earlier stages of cervical cancer (91%, 32/35), and of cancers diagnosed between screening intervals, i.e. interval cancer (60%, 15/25; Figure 11). In women above screening age, 68% (30/44) had advanced stages of cervical cancer, whereas 40% (57/143) of women who did not attend the screening programme had advanced stages. The advanced and localised tumours were most frequently detected by symptoms, whereas microinvasive tumours were mainly detected through the screening programme ($P < 0.001$).

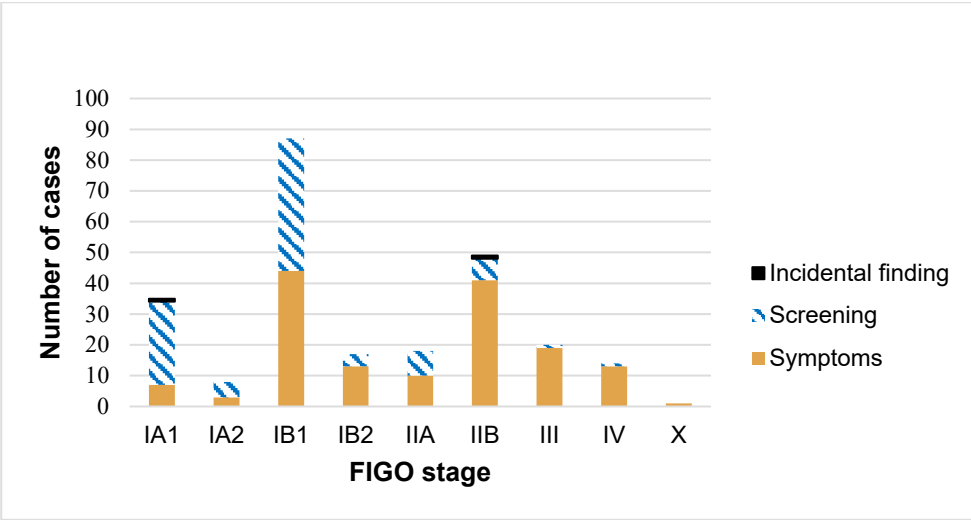

Figure 11. FIGO stage in relation to mode of detection.

Cancer treatment

The most common cancer treatment was radiotherapy and/or chemotherapy, accounting for 109 women in total (44%). In all, 128 women underwent surgery after diagnosis.

Table 4. Treatment modalities in cervical cancer.

Cancer treatment	No. of cases (n)	Percent (%)
Conisation	12	5
Trachelectomy	16	6
Simple hysterectomy	5	2
Radical hysterectomy	69	28
Hysterectomy with chemotherapy and/or radiotherapy	26	11
Radiotherapy and/or chemotherapy	109	44
Palliative care*	6	2
Declined treatment	4	2

**Symptomatic treatment.*

HPV-negative tumours

High-risk HPV was found in 96% (n = 236) of the cervical tumours, whereas 4% (n = 11) were HPV-negative.

Table 5. Characteristics of HPV negative tumours.

Cancer type	FIGO stage	Screening history
Gastric-type adenocarcinoma	IVB	Above screening age
Adenocarcinoma	IVB	Above screening age
Gastric-type adenocarcinoma	IVB	Above screening age
Squamous cell carcinoma	IIB	Above screening age
Adenocarcinoma with focal clear cell features of unknown origin	IB1	Above screening age
Adenocarcinoma, primary endocervical cancer with an aberrant immunoprofile	IB1	Above screening age
Clear cell carcinoma of the cervix	IB1	Above screening age
Gastric-type adenocarcinoma	IB1	Interval cancer
Adenocarcinoma, mesonephric carcinoma originating in the cervix	IVB	Interval cancer
Gastric-type adenocarcinoma	IIB	Interval cancer
Gastric-type adenocarcinoma	IB2	Absence

Paper III

Main findings

Of 1,915 invited participants, 173 women responded. Of these, 101 women had participated in the previous screening round, 37 had not participated, and 45 had a history of cervical dysplasia. The content analysis identified seven categories: (1) unpleasant experiences with a vaginal examination; (2) gratefulness and acceptability of self-sampling; (3) varying perceptions of one's capacity to perform self-sampling; (4) a preference for cervical sampling by healthcare professionals; (5) anxiety and fear concerning a potential or detected HPV infection; (6) different risk assessments for acquiring an HPV infection; and (7) a negative impact on mental well-being due to cervical dysplasia. The overarching theme was that "the HPV self-test reduced practical and emotional barriers to attending the cervical cancer screening programme, but test results may create anxiety."

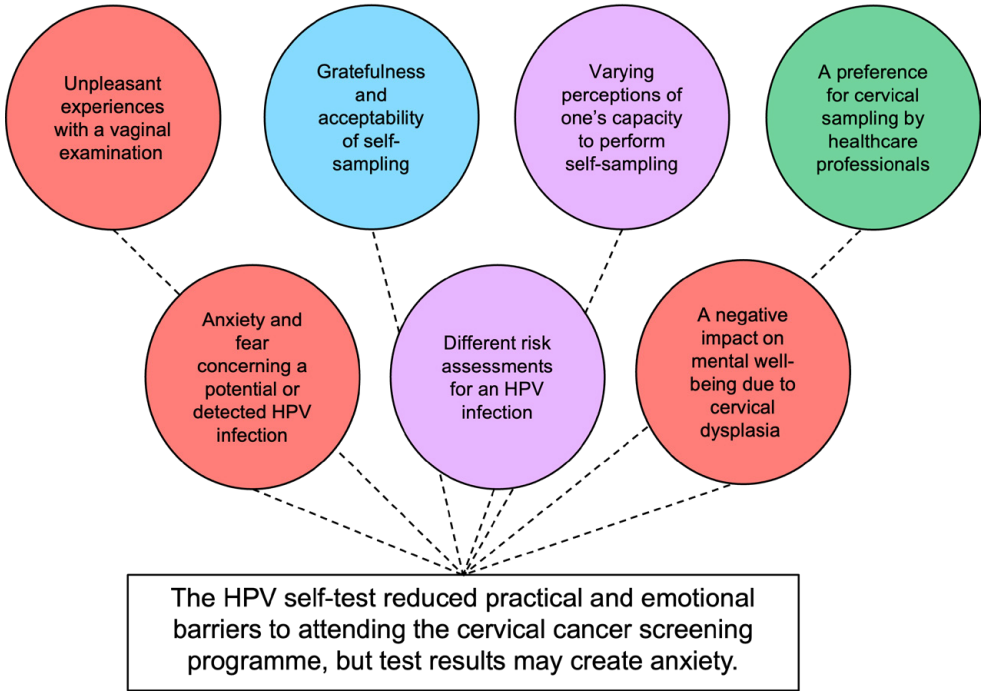

Figure 12. The content analysis resulted in seven categories and were subsequently merged into one overarching theme as presented above.

(1) Unpleasant experience with a vaginal examination

The vaginal examination was described as unpleasant, painful, too intimate, impersonal, and offensive. It was also described as mentally stressful, uncomfortable, awkward, time-consuming, and associated with shame and vulnerability. Women with pain disorders reported a lack of understanding from healthcare professionals.

> *"I definitely prefer self-testing. I find gynaecological visits unpleasant and feel exposed, therefore I have skipped the pap smears for many years… As I have pain problems, the entire visit was painful and left me feeling vulnerable." (95)*

Other reasons that contributed to unpleasant experiences included fear of finding other diseases, unease at not knowing what procedures were being performed, and the fact that someone else was performing the examination. Some women described negative experiences regarding professional care due to a lack of empathy, respect, and understanding.

(2) Gratefulness and acceptability of self-sampling

Trust in healthcare and medical research increased acceptance of the self-test. The information leaflet was described as informative and easy to understand. The self-test was preferred because it was described as easy and time-efficient, with no need for planning and transportation; it was also described as more private, convenient, and practical.

> *"You can do it yourself; you don't have to go anywhere, you don't have to take time off from work, and the best part – it's painless. Many midwives are surprisingly rough." (39)*

Previous traumatic gynaecological examinations, sexual abuse, and anxiety about health also influenced the preference for self-testing. The self-test was described as less stressful and less painful than a pap smear. No unnecessary vaginal examinations were performed, and the women expressed their gratitude for the self-test.

(3) Varying perceptions of one's capacity to perform the self-sampling

Most women believed they had the capacity to perform vaginal self-sampling. However, some women expressed uncertainty and anxiety regarding whether the self-test was performed correctly; consequently, they found it less reliable. Women with rheumatic diseases, tremors, and obesity found it difficult to self-test. When an HPV infection was detected using the self-test, its reported reliability increased.

> *"I would have preferred to do it at the health clinic as it feels safer. It feels uncertain/not as proven to do it at home (with a completely different "brush" as well)." (3)*

Reasons for doubting one's capacity to perform the self-test included uncertainty about how to perform the self-test according to the instructions. Other concerns involved how the sample should be stored and mailed, and the expiration date of the sample.

(4) A preference for cervical sampling by healthcare professionals

Some women said that they valued the personal interaction of a scheduled appointment with a test performed by a healthcare professional because it provided them with the opportunity to receive guidance on problems regarding reproductive health, undergo a regular check-up, and test for other sexually transmitted diseases.

> *"An advantage of having a test taken by healthcare professionals is that you get the chance to ask questions about possible gynaecological problems. Especially in these times when it is incredibly difficult to get hold of a gynaecologist in Region Skåne." (118)*

Another factor that influenced the preference for having a test performed by a healthcare professional was anxiety after treatment for cervical dysplasia. A personal meeting with a healthcare professional was considered safer and more effortless than a self-test. The care provided by healthcare personnel was a positive experience and was described as professional, reassuring, empathetic, friendly, informative, and engaging.

Four women expressed unwillingness to use a self-sampling device. The reasons cited included perceptions that it was inconvenient, unprofessional, and a timesaving and lazy option for the healthcare system. One woman felt affronted to receive the self-test, whereas another was frustrated at receiving it multiple times without actively choosing it.

Most women said that they were pleased with the information on HPV and cervical dysplasia. After detection of HPV and cervical dysplasia, some women perceived the information as inadequate, which led to increased anxiety, symptoms of depression, and a search for answers that were confusing and redundant.

(5) Anxiety and fear concerning a potential or detected HPV infection

Some women expressed feelings of fear, anxiety, sadness, stress, and nervousness about receiving a positive HPV test result. The experience was described as terrifying, and some women reported symptoms of depression. The uncertainty regarding whether the test could detect HPV infection generated anxiety. Asymptomatic HPV infections and the potential development of cervical cancer were also concerning.

> *"Anxiety, fear, and thoughts about the future… However, I had a good doctor, and the process that followed felt safe and reassuring." (36.5)*

Some women did not consider or worry about the test results. Trust in healthcare professionals and the care received, as well as knowing that HPV is a common disease, reduced feelings of anxiety and fear.

(6) Different risk assessments for acquiring an HPV infection

Few women were conscious of the risk of HPV infection, and some had misconceptions regarding the transmission of HPV. Reasons for not reflecting on HPV as a sexually transmitted disease were older age, no sexual contact, or being in a long-term relationship.

> *"Never thought about it, and if you had got it, you know where you've been. Since I am married and have been for many years, I know who to blame." (21)*

(7) Negative impact on mental well-being due to cervical dysplasia

Some women expressed feelings of fear, frustration, stress, anxiety, anger, annoyance, discomfort, shock, nervousness, and depression following the detection of cervical dysplasia. The finding of cervical dysplasia also led to anxiety regarding cancer, death, and future fertility.

> *"I had high-grade cervical dysplasia but no signs of cancer. However, I was afraid that it would lead to the development of cervical cancer. The anxiety was enormous and affected me very negatively… I have started to get used to the thought that I potentially need to defeat cancer and forget my dream to become a mother. My priorities have changed. I have been depressed for over a year. That's how long the examination process and treatments went on. Even on the day I got a negative test result, I couldn't stop worrying about my health." (1)*

The follow-up period was described as stressful, uncertain, and filled with anxiety; some women had a constant fear of reinfection and recurrent cervical dysplasia even after treatment. Decreased mental well-being was attributed to insufficient knowledge, long waiting times, uncertain prognosis, and no existing contact person. Some women initially had feelings of concern and surprise; however, these feelings were replaced by a sense of safety due to the care provided. The knowledge that cervical dysplasia is common and can be treated at an early stage contributed to the absence of negative effects on mental well-being.

> *"My mental well-being is more affected by the tree outside the window that refuses to bloom…" (153)*

Cervical dysplasia and HPV positivity had an impact on the sex lives of some participants. Some women described feelings of being "dirty," "disgusting," and "limited." After a positive HPV result, some women reported that it was mentally harder to have sex; their sex drive decreased, and for one woman, it disappeared. HPV positivity was considered proof that their partner had been unfaithful. For women who had not been sexually active in a long time, a positive HPV result was shocking. HPV positivity and cervical dysplasia also led to a more restrained approach to sex and regret concerning earlier sexual contact.

Table 6. Women's characteristics.

	No. of cases (n)	%
Participation in the screening programme		
Regular attendance	101	73.2
Absence	37	26.8
Level of education		
Primary education	9	5.4
Secondary education	35	20.8
University degree	124	73.8
Native language		
Swedish	140	83.4
Smoking		
Yes	9	5.4
Sometimes	15	9.0
No	142	85.5
Sexual partners		
0-9	109	65.7
10-19	33	19.9
20-29	14	8.4
> 30	10	6.0
Pregnancies		
0	33	19.8
1	18	10.8
2	52	31.1
≥ 3	64	38.3
No contraceptives	116	69
Use of contraceptives	52	31
Condom	18	34.6
Contraceptive cap	1	1.9
Mini pill (progesterone only pill)	4	7.7
Combined oral contraceptive pills	5	9.6
IUD (hormonal coil)	19	36.5
IUD (coil)	5	9.6
Relationship status		
In a heterosexual relationship	111	66.5
In a same-sex relationship	9	5.4
Single and no sexual relationships in the last 6 months	36	21.6
Single with sexual relationships in the last 6 months	11	6.6

Paper IV

HPV positivity in self-samples

From September 1, 2021, to December 31, 2024, the screening programme distributed 557,976 vaginal self-sampling kits. A total of 208,386 were returned, giving a participation rate of 37% (95% CI: 37.22–37.47%). Invalid tests were recorded for 3,623 women (1.7%), who were subsequently referred for midwife-collected cervical samples. Among the remaining valid tests (n = 204,763), HPV was detected in 39,697 women, corresponding to a prevalence of 19% (95% CI: 19.22–19.56%) (Figure 13).

Follow-up with cervical samples

Of the HPV-positive group, 32,305 women (81%) attended a follow-up visit at a midwife clinic through the end of the fourth subsequent calendar month (Figure 13). The mean interval between the initial self-sample and the follow-up cervical sample was 83.4 days (SD: 21.7), with a median of 85.0 days (range: 0–144). At follow-up, 39% (12,495/32,305) of the women were still HPV-positive. Within this group, HPV16 was found in 8.5% (1,063/12,495) and HPV18/45 in 8.8% (1,094/12,495).

Cytology assessment

Cervical cytology results were assessed in 12,505 women, as shown in the table below. The total number of cytology results exceeded the number of HPV-positive cervical samples by 10. This discrepancy is explained by nine women who had two different cytological diagnoses (originating from squamous and glandular cells) within the same sample, and one woman with two cervices. In our study, a high prevalence of ASCUS (50%) and LSIL (33%) was observed, while benign cytology results were reported in 10% of women.

Table 7. Reflex cytology results among the HPV-positive women following cervical sampling.

Cytology result	n	%
Benign	1,300	10
ASCUS	6,261	50
AGC	36	0.3
LSIL	4,114	33
ASC-H	419	3.4
HSIL	340	2.7
Suspicion of adenocarcinoma	18	0.1
Suspicion of squamous cell carcinoma	4	0.0
Insufficient sample, non-diagnostic	13	0.1
Total	**12,505**	**100%**

ASCUS = Atypical Squamous Cell of Undetermined Significance. AGC = Atypical glandular cell. LSIL = Low-grade Squamous Intraepithelial Lesion. ASC-H = Atypical Squamous Cells, cannot exclude High-grade squamous intraepithelial lesion. HSIL = High-grade Squamous Intraepithelial Lesion.

Histopathological assessments

During follow-up with gynaecologists, 7,647 biopsies or loop electrosurgical excision procedure specimens were assessed through the end of the 12th subsequent calendar month of the cervical sampling date. Of these, 6,070 (79%) were performed through the end of the sixth subsequent calendar month. The histopathology results are shown below.

Table 8. Histology results among the HPV-positive women at cervical sampling with biopsy or cone specimens at colposcopy.

Histology result	n	%
Benign	4,288	56
Cellular atypia	219	2.9
LSIL	1,857	24
ASC-H	8	0.1
HSIL	923	12
AIS	47	0.6
CIS	2	0.0
Squamous cell carcinoma	25	0.3
Adenocarcinoma	6	0.1
Adenosquamous carcinoma	1	0.0
Unsatisfactory specimen	183	2.4
Unclear	88	1.2
Total	**7,647**	**100%**

LSIL = Low grade squamous Intraepithelial lesion. ASC-H = Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion. HSIL = High grade squamous Intraepithelial lesion. AIS = Adenocarcinoma in situ. CIS = Cancer in situ

Cervical cancers

During the study period, the screening programme identified 32 cases of cervical cancer through HPV self-sampling. The most common histological type was squamous cell carcinoma (n = 25), followed by adenocarcinoma (n = 6) and adenosquamous carcinoma (n = 1). According to the International Federation of Gynaecology and Obstetrics classification, microinvasive (stage IA1) and localised tumours (stage IA2–IB2) were significantly more common (n = 24) than advanced-stage tumors (stage IB3–IV; n = 7) (p = 0.003). One tumour was unstaged due to non-participation in follow-up procedures. In addition, 69% (22/32) of women diagnosed with cervical cancer had a history of irregular screening participation. Most cancer cases were associated with high-risk HPV, particularly HPV16 and HPV18/45.

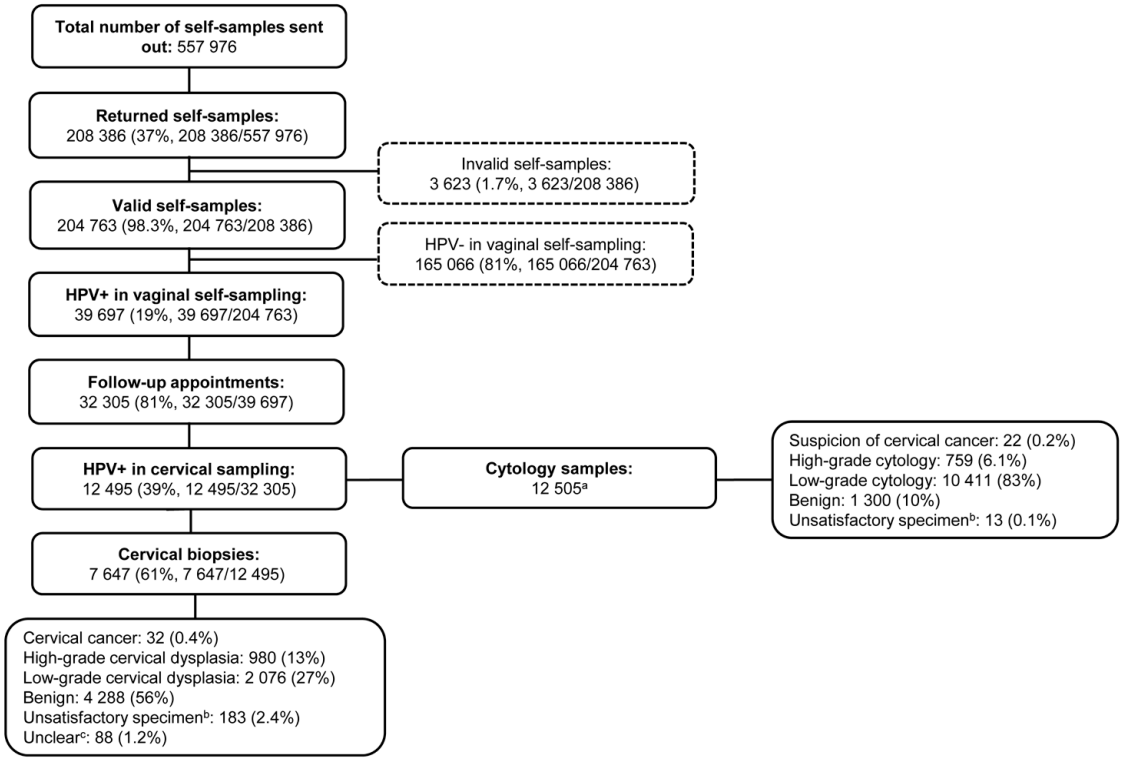

Figure 13. Stepwise outcomes in HPV-based cervical cancer screening with vaginal self-samples. Samples were collected from Region Skåne during September 2021-December 2024 with follow-up until April 2025. HPV+ = HPV positive sample. HPV- = HPV-negative sample. High-grade cytology includes diagnoses such as ASC-H (Atypical Squamous Cells, cannot exclude High-grade squamous intraepithelial lesion, n= 419), and HSIL (High-grade Squamous Intraepithelial Lesion, n = 340). Low-grade cytology includes ASCUS (Atypical Squamous Cell of Undetermined Significance, n = 6,261), LSIL (Low-grade Squamous Intraepithelial Lesion, n = 4,114), AGC (Atypical glandular cell, n= 36). High-grade cervical lesions in biopsy sampling includes ASC-H (n = 8), HSIL (n = 923), AIS (n = 47), and CIS (n = 2). Low-grade cervical lesions includes cellular atypia (n = 219) and LSIL (n = 1,857). [a]There are 10 more cytology results than HPV-positive cervical samples. This discrepancy is explained by nine women having two different cytological diagnoses from the same sample (originating from squamous and glandular cells), and one woman having two cervices. [b]Unsatisfactory specimen refers to materiel being too sparse for analysis. [c]Unclear refers to no diagnosis.

Discussion

Screening history of women with cervical cancer

In high-income countries, including Sweden, cervical cancers generally occur in women with a history of inadequate screening, while screen-detected tumours frequently reflect suboptimal management or follow-up of abnormal results (18). Paper II showed that the screening programme detected 39% of the cervical cancer cases, and about six of 10 women were diagnosed through symptoms. The most common screening history in women with cervical cancer was irregular screening (143, 57.9%), followed by women being above screening age (44, 17.8%). A case-control audit in Sweden during 2002–2011 showed that women with no screening history over the past two screening rounds before diagnosis had a fourfold increased risk of cervical cancer compared with women who were screened regularly. The more advanced stages of cervical cancer were also more prevalent among unscreened women, with a 2.5-fold increased risk. Women diagnosed through symptoms compared with screen-detected cervical tumours, had a 19-fold increased risk of more advanced stages at diagnosis (18). We also found a linkage between higher FIGO stages and discovery due to symptoms, which is in accordance with earlier studies (17, 136). Patients with screen-detected tumours have shown significantly improved overall survival, in contrast to patients with tumours detected due to symptoms (136, 137).

In our data, we also show that a substantial proportion of women were diagnosed after exiting the screening programme (n = 44, 17.8%). Two-thirds of the women above screening age were diagnosed with cervical cancer under the age of 80. In Region Skåne, the mean life expectancy is approximately 84 years (138), indicating that many women after the last screening round at age 64–70 still have several years where they are susceptible to cancer development. A reactivation of an HPV infection can occur after a long period of viral latency (53), and elderly women with an active sex life might also find new partners later in life, which increases the risk of acquiring an HPV infection. This result is in line with another study conducted in Region Skåne which showed that elderly women (>65 years of age) are more often diagnosed with advanced stages of cervical cancer, with a median survival time after diagnosis of approximately three years (136). The cure rate for cervical cancer is highly dependent on the FIGO stage; hence, extending the age limit for the last screening round could be considered. Of the 44 women above screening age,

only two had undergone HPV testing before their cancer diagnosis, and both tested positive. Among older women, a negative HPV test is associated with an 84% lower risk of developing cervical cancer compared to those with only a benign cytology result. Furthermore, the incidence rate of cervical cancer with a negative HPV test was 3.0 per 100,000 person-years, versus 18.8 per 100,000 person-years among those with negative cytology assessments (139). These findings underscore the importance of including HPV testing as part of the exit screening strategy. After the implementation of primary HPV screening in 2017, the incidence of cervical cancer should decrease in women above screening age when HPV-positive women are followed up and treated for precancerous lesions.

In study IV, microinvasive (stage IA1) and localised tumours (stage IA2–IB2) were significantly more common (n = 24) than advanced-stage tumours (stage IB3–IV; n = 7) (p = 0.003). Notably, the majority of women diagnosed with cancer had also participated in cervical screening irregularly (69%, 22/32). In our audit, attendance at the screening programme detected earlier stages of cervical cancer (91%), and of cancers diagnosed between screening intervals, i.e. interval cancer (60%). The distribution of interval cancer was relatively equal for women >50 years of age and women <50 (P = 0.83), indicating that longer screening intervals in women >50 do not increase the risk of cervical cancer. Therefore, it is reasonable to consider HPV testing every five years as an appropriate screening interval for both women aged 23–49 and women aged 50–69. This approach would ensure adequate detection and monitoring of cervical cancer risk in both age groups. The high NPV of testing HPV-negative in routine screening, offers a potential for extended screening intervals (62, 139, 140). The new national screening guidelines recommend extending the screening interval to five and seven years in the previously mentioned age groups. As described in the introduction of this thesis, current WHO guidelines recommend an mRNA-based screening interval with cervical samples of no more than five years, since accumulated evidence is heterogeneous and limited. Further research is therefore needed to establish the long-term safety of extending screening intervals beyond five years with the Aptima mRNA assay on self-samples (22).

In our audit, we found 21 (8.5%) women with cervical cancer and a history of high-grade cervical dysplasia who had been adequately followed up. Women treated for cervical dysplasia with HPV-negative follow-up samples have a 1.4% (95% CI 0.9% – 2.1%) risk of CIN2+ at a later follow-up (141). To detect residual AIS, HPV analysis is also the most sensitive method (12). After an excision for HSIL/AIS with HPV-negative follow-up, the risk for a persistent or new HSIL/AIS has been shown to be 3.7%; using double testing with negative HPV and cytological analysis, the risk is 2.4%; and with two negative HPV tests, the risk is less than 3% (142). Several screening programmes advocate double testing as a test of cure after treatment of HSIL or AIS, as well as shorter surveillance intervals. Fortunately, after conisation treatment, very few patients remain HPV-positive with a subsequent risk of cervical cancer (143). However, these women remain at risk for future disease. Compared to

the general screening population, women previously diagnosed with CIN3+ have a more than twofold higher risk of developing cervical cancer (144). Our results indicate that the patients with a screening history of high-grade cervical dysplasia who later develop cervical cancer account for less than 10% of the total number of women diagnosed with cervical cancer in this study. However, as in primary screening, adherence to follow-up after treatment for cervical cancer is crucial. Special efforts should be made to ensure that a test of cure (HPV analysis with cytology) is performed following treatment in order to minimise the risk of subsequent cervical cancer.

Primary HPV screening

In our audit, a normal screening history prior to cancer diagnosis was seen in 52% (129/247) of the women. The vast majority of patients with interval cancer had a normal screening history (85%, 21/25). A study in Sweden found an increase in normal cytological screening results prior to diagnosis of invasive cervical cancer in 2014, suggesting decreased protection from a benign cytology result due to inadequate cytological diagnosis. The largest increase was observed for adenocarcinoma, representing a 31% rise (107). This may, to some extent, be solved with HPV screening, which has proved to have a higher sensitivity compared with cytology, and is more effective than cytology in preventing adenocarcinoma (12). As mentioned earlier, the majority of women above screening age in our audit had no record of an HPV test before exiting the screening programme. For postmenopausal women, HPV testing demonstrates higher sensitivity than cytology (145, 146), although the sensitivity of HPV testing also decreases with age (147). The transformation zone is situated higher up in the endocervical canal due to hormonal changes, making adequate visualisation and sampling more difficult during colposcopy in elderly women. In addition, age-related vaginal atrophy and cervical stenosis further contribute to increased discomfort and technical challenges during pelvic examinations (148). Vaginal self-sampling does not offer cytology performance, but methylation markers (35), microRNA expression (149), next-generation sequencing (150), or extended genotyping may improve the prediction of women at higher risk for cervical neoplasia and reduce the number of unnecessary colposcopies (34).

The cumulative incidence rate of CIN3+ after six years has been shown to be 34% among women with abnormal cytology and HPV positivity. Among women with normal cytology but a positive HPV test, the cumulative incidence rate reached 10% over the same period. In contrast, a lower incidence of 2.7% was observed in women with HPV-negative test results but with an abnormal cytology assessment, and an even lower risk of 0.28% was found in women with both negative cytology and negative HPV testing (151). Since September 2021, the self-sampling approach has

been implemented for all women invited to the screening programme in Region Skåne. The first round of HPV-based screening typically detects a higher number of precancers and cancers, largely due to the identification of cases previously missed by cytology. A declining trend in detection rates is then generally observed in subsequent screening rounds (152). In 2017, the cancer incidence in Region Skåne was 11.37 per 100,000 women and in 2018, 14.31 per 100,000 women. Thereafter the incidence has dropped and in 2023 the overall cervical cancer incidence rate in Region Skåne was 9.68 per 100,000 women, compared to the national incidence rate of 11.64 per 100,000 women (153). The slightly lower cervical cancer incidence in Region Skåne may be attributed to the effectiveness of the self-sampling programme and subsequent follow-up, facilitating early detection and treatment of precancerous lesions. However, since 2017, the incidence of cervical cancer in southern Sweden has consistently been lower than the national average, coinciding with a higher screening attendance rate, which may have contributed to the reduced cervical cancer incidence (154). The enhanced sensitivity of HPV screening has not been shown to result in overdiagnosis during long-term follow-up but is more likely explained by the earlier detection of precancerous lesions (32).

Vaginal HPV self-sampling

The self-sampling approach offers a means to increase screening coverage, thereby facilitating earlier detection of precancerous lesions, which can be effectively treated. To maximise the impact of cervical cancer screening programmes, it is essential to ensure that they are efficient, accessible, and equitable. As previously described in Paper II, most cervical cancers occurred among women who had been inadequately screened. Several studies have demonstrated that offering self-sampling kits to non-attendees of cervical screening improves attendance (15, 16, 155, 156). Among women who regularly attend the screening programme, a high level of acceptability for self-sampling has also been established (157, 158).

The participation rate was 33.5% in the self-sampling arm and 47.5% in the control arm ($p < 0.0001$) in Paper I, and 37% for self-samples in Paper IV. Gustavsson et al. conducted a similar study in Uppsala in 2013-2015 for women aged 30–49 years and showed that the participation in the self-sampling HPV arm was slightly increased (47%) compared to the cervical control arm (39%) (159). The lower participation observed in Paper I may partially be explained by the fact that no reminder was sent to women who failed to submit their HPV self-sample. In the Netherlands, hrHPV-based screening was implemented in 2017. Between January 2017 and June 2018, the programme included 454,573 women who were eligible for HPV-based screening, and 8% chose the self-sample device, and the total combined participation rate was 61% (160). The participation rate increased slightly

in Paper IV to 37%. The compliance, measured over one screening interval plus six months, was 83% in 2022-2023, aligning with previous years but showing a slightly higher coverage rate (161). No short-term reminders were sent to women; had this been done, it may have improved adherence to the self-sampling device. After one year, a second self-sampling kit was sent to those who had not returned the initial kit. In our studies, the overall lower participation in conducting the self-sampling test may have been due to resistance to the switch to self-sampling since it is not yet recognised and trusted by all women. A higher return rate of self-samples has been observed in the Stockholm–Gotland region of Sweden, where self-sampling was introduced earlier and implemented more extensively. In this region, return rates have reached approximately 50% (personal communication), likely reflecting both greater public familiarity with the method and a more established infrastructure for self-sampling initiatives. The compliance rate for HPV self-sampling is expected to increase as awareness and acceptance of the method become even more widespread. In the meantime, healthcare professionals and screening programme authorities play a pivotal role in facilitating its acceptance and integration into routine screening practices by educating women about the procedure and its safety. When updating screening protocols based on new scientific evidence, it is essential to effectively communicate the rationale behind these changes to the screening population. Clear communication helps ensure that the modifications are better understood and accepted. A study in Australia evaluated women's views about the renewed screening programme, implemented in December 2017, with extended screening intervals from two to five years. Six focus groups showed that the knowledge of HPV among women was low, even for younger women who were HPV-vaccinated (162). Knowledge about HPV in the general population has been shown to be limited (163), with misunderstandings regarding HPV testing among the screening population (164). Women's primary concern about the extended screening intervals was the risk of missed cancers (162).

Previous studies have identified several reasons for non-attendance, including embarrassment (165, 166), fear of pain (165), anxiety about potential test results (165), forgetting the appointment (165, 166), lack of time (167), previous negative experiences with cervical samplings by midwives (166, 167), and a perception of being healthy (167). Most of these practical and emotional barriers to participation may be addressed through the use of self-sampling devices. Overall, in Paper III, women said that they appreciated preventive measures and felt positive and grateful for the HPV self-sampling device. The preference for the self-test was attributed to its efficiency, convenience, ease of use, and privacy. These findings are consistent with two earlier studies that explored women's experiences with the self-test device (157, 168). Vaginal examinations were described as mentally and physically stressful by some women in this study. The self-sampling device offers a potential solution that can address practical challenges and improve screening participation rates. We also found a preference for the self-sampling device among women with

prior traumatic gynaecological experiences or sexual abuse, allowing them to overcome emotional barriers to attendance.

Only a few women in this study lacked confidence in their ability to perform the self-tests; the primary reason was their preference for a qualified professional to conduct the sampling. Sultana et al. reported that 81% of women who were under-screened or had never been screened expressed confidence in their ability to perform a self-sampling test correctly. However, 57% of these women reported uncertainty regarding the accuracy of the test (16).

The highest proportion of returned samples came from women aged 50–64 in the self-sampling group, whereas the largest proportion for the cervical samples was from younger women aged 30-49 years (Paper I). The same trend was seen in a previous study by Kellen et al. (169). Logistical problems and discomfort may discourage older women from attending a gynaecological examination (170). It is also possible that younger women have higher anxiety levels concerning their sexuality and fertility and, therefore, need personal counselling, especially given that HPV prevalence is also greater in this younger age group. However, in Paper IV, our results indicate that the older the women were, the less interested they were in performing self-sampling. A possible explanation for this may be that older women are more likely to prefer continuing with a well-established routine and are generally less accustomed to adopting new health innovations compared with younger women. Furthermore, information about self-sampling may not have reached older women as effectively, and self-sampling may also be perceived as more practically challenging in this group. In Paper III, some women (n = 20) expressed a preference for a clinician to collect cervical samples. The median age of these women was 47 years. They highlighted the importance of personal contact with healthcare professionals and valued the opportunity for a general check-up during which they could also ask questions related to their reproductive health. However, it is important to emphasise that the primary aim of the cervical cancer screening programme is the prevention of cervical cancer, rather than the provision of general gynaecological examinations or consultations regarding broader health concerns.

High compliance (91.2%) for clinical follow-up among self-sampling HPV-positive women was observed in Paper I. Our results align with previous studies involving both attenders and non-attenders in the screening programme, demonstrating high compliance with follow-up procedures following the detection of HPV in self-collected samples (160, 171-174). The attendance rate for follow-up appointments was, however, lower than expected in Paper IV (81%). The 19% loss to follow-up observed after an HPV-positive test result in this study is an important finding that warrants further evaluation. This level of attrition may reflect limited understanding of the significance of an HPV-positive result among women in the screening population, and the information provided in the notification letters following an

HPV-positive result might be insufficiently clear, underscoring the need for improved communication strategies.

HPV prevalence

Our findings from Papers I and IV indicate higher HPV positivity rates, 17.1% and 19%, respectively, compared to DNA-based HPV analyses (158, 175). One potential explanation for the substantially higher HPV prevalence in our studies is the effect of a pre-heating step prior to the analysis of self-collected samples. This procedure has been shown to increase assay sensitivity (75) and was introduced in the southern region of Sweden on January 17, 2019. The rationale for this modification was based on earlier findings that the Aptima mRNA assay, when applied to self-samples, demonstrated lower sensitivity for detecting CIN2+ lesions (0.84 [95% CI 0.74–0.96]) compared to first-generation comparator tests in a meta-analysis conducted by Arbyn et al. (66). Currently, all self-samples are pre-heated at 90°C for 1h 15 minutes before analysis. The primary task of the Aptima assay is to detect E6/E7 mRNA transcripts of HPV-infected cells; however, HPV DNA can also be detected (77). The pre-heating step in our method probably results in an increased number of single-stranded HPV DNA molecules, which are then captured with the Aptima. In PCR-based methods, DNA is separated into single-stranded templates at temperatures around 95°C (78). mRNA is a less stable molecule and starts degrading at temperatures around 40°C (79). However, in the protocol developed by Forslund and Borgfeldt, the pre-heating step did not appear to degrade HPV mRNA in the Aptima (STM) solution. In three of the five originally HPV-positive samples, the HPV S/CO values increased significantly following incubation at 90°C for one hour. The authors suggest that it is possible that heat treatment may disrupt molecular interactions between vaginal tract components and HPV mRNA, thereby enhancing the sensitivity of the assay when applied to vaginal self-samples (75). This phenomenon warrants further investigation to clarify the molecular integrity and diagnostic relevance of heat-treated samples. Such evaluations should also adhere to the VALHUDES (VALidation of HUman papillomavirus assays and DEtection Systems) protocol (80) and should test for non-inferiority of the Aptima mRNA assay on self-sampling including the pre-heating step compared to DNA-based methods in a real-world setting.

Increased detectability of HPV in vaginal self-samples may also partially reflect HPV infections only localised to the vagina and not the cervix. HPV detection in the vagina may be due to shedding from the cervix or may reflect non-progressive infections. Spontaneous HPV clearance of the cervix may also explain disparities in HPV prevalence between the self-sampling and the follow-up cervical samples. Notably, about two-thirds of the self-sample HPV-positive women had no detectable HPV in

the cervical sample at the clinical follow-up in studies I and IV. In a study by Gustavsson et al., repeat vaginal HPV testing was performed on average 4.4 months after an initial HPV-positive test result and demonstrated that approximately 30% of women had cleared their HPV infection (159). In two recent studies, HPV self-sampling kits have been evaluated with the Aptima mRNA assay and the pre-heating step (68, 176, 177). In a study conducted in Scotland, concordance between the first and second vaginal self-samples for non-attenders at follow-up was 67.1%, and for the initial vaginal self-sampling test and the clinician-collected cervical sample, the concordance was 30% (176). The performance of the Aptima mRNA assay in paired self-collected and clinician-collected samples of postmenopausal women was assessed in Örebro Region, Sweden. HPV was detected in 284 cases in the vaginal samples and in 77 cases in the cervical samples. Importantly, all cases of HSIL (n= 7) were detected with both self-sampling and cervical-collected samples (68). Previous studies have also reported a higher prevalence of HPV in vaginal samples compared to cervical samples. Ketelaars et al. demonstrated that while the proportions of HPV16 and HPV18 were similar in both vaginal and cervical samples, non-HPV16/18 types were more frequently detected in self-collected vaginal samples (158). Since endocervical cells represent the main site for high-risk HPV infection and exhibit the highest viral load relative to the vagina, a highly sensitive test is necessary to maintain comparable detection accuracy between clinician-obtained and self-collected samples (74). Increased sensitivity of self-samples has been observed when a smaller sample volume is used with the self-sample device as compared to the larger cervical sample volumes of 20 mL, likely due to the increase in cellular concentration (83).

In Paper I, there were no invalid HPV tests in the control arm, whereas 3.7% (n = 184) of self-samples were invalid. Some of these did not have sufficient sample volume for the HPV assay, while others showed invalid test results due to internal control failures of the samples. In Paper IV, 1.7% (n = 3,623) of the self-samples were deemed invalid. A possible explanation for the internal control failures could be that the transport medium used for the self-sampling differs from the liquid used in the cervical sampling. To minimise the risk of invalid samples, the HPV analysis must be performed within three days after the pre-heating step. In study IV, we observed that the proportion of invalid self-samples increased with age. Among women aged 50–70 years, 4.6% of the self-samples were deemed invalid, compared with 0.6% among women aged 30-49 years and 0.4% among women aged 23-29 years.

Among the cervical tumours, 4% (n = 11) were HPV-negative (10 LBC samples analysed by Aptima and one FFPE sample analysed by Luminex), and only one woman had a previous record of an HPV test. There might have been an HPV infection initiating the onset of cervical dysplasia, and as it progressed to invasive cervical cancer, the HPV genome might have been lost. Since nine of 11 tumours were adenocarcinomas, and all women had a normal screening history, this indicates that cytology has an inferior sensitivity in detecting adenocarcinomas. A global study on HPV genotype distribution among patients with invasive cervical cancer

demonstrated that HPV-negative tumours were more frequently identified in cases of adenocarcinoma compared to other histopathological subtypes of invasive cervical cancer (178). Even though a persistent HPV infection is the main causative agent for the progress of invasive cervical cancer, some tumours develop without any detectable HPV (179, 180). The HPV-negative tumours have been shown more often to be non-squamous carcinomas, diagnosed at more advanced stages of cervical cancer, more frequently detected in elderly women, and with a worse overall survival rate (179), which is in line with our results. The median age for women with HPV-negative tumours was 71 compared with the overall median age of 52. An age-related increase in HPV-negative tumours was also reported in a meta-analysis by Hammer et al., indicating that the loss of HPV during cancer progression or age-related genomic alternations may contribute to cervical cancer development in older women (181). Possible explanations for HPV negativity in our study might be that tumours were not derived from an HPV infection, there was degradation of HPV in the tumours due to necrosis, or misclassification of cervical cancer. Errors in the sampling method are another possible explanation for HPV negativity. Even though the Aptima assay has an internal control mechanism for overseeing the three steps of target capture, amplification and detection, it does not use a human housekeeping gene to control for inadequacy. Thus, sufficient acquisition of tissue material for analysis is not guaranteed (72). It is inevitable that some cases of cancer will be missed within the framework of a screening programme. This is particularly concerning in relation to truly HPV-negative tumours, which are not detectable through primary HPV-based screening. In our audit, among the 11 HPV-negative tumours identified, five were classified as gastric-type adenocarcinomas, one as clear cell carcinoma, one as adenocarcinoma with focal clear cell features of unknown origin, one as mesonephric adenocarcinoma, and one as endometrioid adenocarcinoma. According to the 2020 WHO Classification of Female Genital Tumours, these subtypes are recognised as HPV-independent (182), and are associated with poorer overall survival outcomes (183).

Cytology assessment

In Paper I, 18.5% of samples with normal cytology were HPV-positive at follow-up, whereas in Paper IV, the corresponding proportion was 10.4%. This is a large difference from the organised cervical screening programme in our county in 2017 where 3.9% of the normal cytology samples were HPV-positive at primary HPV screening (6). In the self-sampling group, both HPV testing and cytology were performed for all women who attended the follow-up visit. In the control group, however, only cytology was performed in cases with a positive HPV result, as per routine practice. The cytotechnologists were aware of the HPV-positive status in the LBC samples before to conducting the cytological analysis. It is likely that the

cytotechnologists were influenced by knowledge of the HPV result and, to a greater extent, classified HPV-positive women as having ASCUS rather than benign cytology. It could also be argued that a healthier population was observed in the self-sampling arm compared to the control arm.

In Paper IV, cervical cytology results demonstrated a high prevalence of ASCUS and LSIL (83%, 10,371/12,505), findings typically associated with transient HPV infections. Our results from southern Sweden represent the highest proportion of low-grade cytology compared with other laboratories in the country (personal communication). The reason for this marked increase is unknown. It is unlikely that these findings reflect a true population-based difference compared with other regions in Sweden; rather, they are more likely to indicate overdiagnoses which may lead to unnecessary referrals for colposcopy and eventually conisation treatment. The increased number of referrals to colposcopy units for ASCUS+ cytology results leads to inefficient allocation of health resources, failing to ensure that efforts are directed where they yield the greatest public health benefits, and resulting in a cost inefficient strategy. To reduce overtreatment and minimise the associated harms, robust quality assurance is essential.

Among women diagnosed with cervical cancer in study IV, prior cytology revealed CIN2+ lesions in 79% (12/15) of those aged 50–70 years, in 92.7% (12/13) of those aged 30–49 years, and in 100% (4/4) of those aged 23–29 years. In women aged 50-70, 105 women had histologically confirmed high-grade dysplasia, and cytology revealed low-grade abnormalities in 52%, high-grade in 46%, and suspected adenocarcinoma in 2%. These findings highlight cytology's limitations in accurately reflecting disease severity, especially in older women.

Histopathological assessment

The vaginal self-sampling approach detected a similar proportion of severe cervical dysplasia as regular midwife-collected cervical screening, 0.48% (95% CI, 0.3–0.72%) in the self-sampling group and 0.47% (95% CI, 0.3–0.66%) in the cervical sampling group, respectively. As both groups were comparable in age and time of invitation, invitation bias is eliminated. Women aged 40–42 were excluded from the self-sampling arm since they should be tested both for HPV and cytology at baseline screening according to national guidelines. In non-attendees to the cervical screening programme who received a self-sampling device in the same county, the prevalence of HSIL and AIS was in 2017 found to be 1.3% (184). The coverage ratio of the screening programme in southern Sweden is higher compared to the national coverage ratio; this might, to some extent, influence the lower rate of cervical dysplasia and cancer incidence in our region. The incidence of cervical

cancer in Sweden varies significantly across regions, with the highest reported incidence in Halland at 16.22 per 100,000 person-years and the lowest in Norrbotten at 4.28 per 100,000 person-years in 2022 (185). In Paper IV, biopsy-confirmed high-grade cervical dysplasia or cancer was identified in 0.5% of all eligible self-collected samples (1,012/204,763), a proportion consistent with that observed in Paper I.

Psychological aspects

The benefits of the cervical cancer screening programme must outweigh the harms. An increasing number of women will test HPV-positive with the self-sample device, and it is consequently important to evaluate the psychological aspects of testing HPV-positive. Anxiety and fear of an HPV infection were described in Paper III. Adverse psychological reactions to a positive HPV result have been reported in previous studies (131-133). Maissi et al. found three variables that predicted higher levels of anxiety: younger age, lack of understanding of smear test results, and women who perceived their own risk of developing cancer as being higher (186). In contrast, long-term anxiety after testing positive for HPV was not observed in two studies (134, 135). The women in the present study acknowledged the importance of attending follow-up appointments after a positive HPV result. Thus, the initial barrier to participation in a screening programme involving gynaecological examinations may be mitigated following an HPV-positive self-sampling result.

The psychological aspects of cervical dysplasia for women referred for colposcopy have been evaluated in a previous study in the southern part of Sweden. The results showed that the state anxiety levels and depression scores were higher among women referred for colposcopy compared to women who only had their pap smear taken in the screening programme ($p < 0.001$, and $p = 0.004$, respectively). A high depression test scores (MADRS-S $\geq$ 12), was identified as the most significant risk factor contributing to elevated levels of state anxiety. This study also emphasises the need for explanation and reassurance at the colposcopy clinic as a statistically significant reduction in the state anxiety after examination was seen, although not for women with severe depression (187). Another study conducted interviews with 15 women referred for colposcopy in 2021-2022. The results revealed that women had a poor understanding of their self-perceived risk of cervical cancer, and searching for more information online and waiting to be referred for colposcopy generated anxiety (188). During the implementation of the self-sample device in 2021, most enquiries from women participating in the screening programme in the southern part of Sweden were about the sampling process itself. However, by 2022, the most common reason for contacting the coordinator was related to testing HPV-positive, with individuals wanting a prompt follow-up appointment with a midwife.

This highlights the importance of giving sufficient information about HPV and the rationale behind the screening protocol.

Strengths and limitations

Paper I

The strength of this study is the large number of women recruited in the self-sampling and cervical sampling groups, indicating that it is scalable for the general population in Sweden. HPV analysis is an objective method compared to the subjective cytology, and thus avoids bias. When cytology was performed at the follow-up, the cytologist knew in both arms that the HPV result was positive. The laboratory used the same assay for all specimens. Women with hrHPV positivity showed high adherence to follow-up visits. The prevalence of HSIL and AIS measured 0.48% in the self-sampling arm and 0.47% in the cervical sampling arm. Invitation bias is minimised, as both groups are comparable with respect to age and timing of invitation. In non-attendees to the cervical screening programme who received a self-sampling device in the same county, the prevalence of HSIL and AIS was in 2017 found to be 1.3% (184). The restricted time period in which our study was conducted and the lower participation rate in the self-sampling arm should be addressed in further studies and regular screening programmes. In retrospect, an evaluation of the Aptima assay's diagnostic performance through direct comparison of self-collected and clinician-collected samples could have added further value to this study.

Paper II

Strengths of this study include the assessment of real-world outcomes following the transition from cytology-based screening to primary HPV screening in southern Sweden. Data were obtained from both national and regional cancer registries, thereby minimising the risk of missing cancer cases within these databases. Limitations include the potential for incomplete or missing screening histories for some women, possibly due to relocation, opting out of national registers, or receiving care in the private healthcare sector. As a quality assurance audit conducted within the Swedish healthcare context, the findings may have limited generalisability to settings with different screening protocols or healthcare infrastructures.

Paper III

A strength of this study is its use of maximum variation sampling (125), including regular attenders, non-attenders, and women with cervical dysplasia in various age groups and cities in Region Skåne. Credibility was further increased by investigator triangulation during the analysis process, which was conducted by three researchers with diverse backgrounds, knowledge, and experience related to the study question (128). The response rate among non-attenders was low (2.5%), as predicted; however, additional contributing factors to non-participation may include the time required to complete the questionnaire and paperwork fatigue. Notably, this study was conducted in a Scandinavian context where there was publicly funded free-of-charge cervical screening; nonetheless, the findings should be generalisable to high-income countries with good insurance-based healthcare. This study has some limitations. First, the screening test results could not be verified because the questionnaire was anonymous. Second, the women may have overestimated or underestimated the time lapse since their last screening test; therefore, the self-reported estimates of their screening programme attendance should be interpreted with caution. Third, women with low literacy may have been underrepresented in the questionnaire responses as most respondents were well educated. However, the questionnaire was available in both Swedish and English to minimise the exclusion of non-native Swedish speakers.

Paper IV

This large-scale, population-based study involving over 557,000 distributed self-sampling kits offers robust and generalisable insights into the real-world implementation of vaginal HPV self-sampling within an organised screening programme. Key strengths include standardised laboratory procedures, centralised analysis, and comprehensive data collection from validated systems, ensuring high data quality. Clinically relevant outcomes were assessed, including cytological and histopathological findings. Although only 37% of women returned self-samples, this should be viewed in the context of an overall screening coverage of 83-85% over a complete screening interval. Targeted interventions—such as automated reminders, clearer communication, and streamlined follow-up access—could enhance compliance. Limitations include potential selection bias, as responders may have been more health-conscious than non-responders, and the absence of a comparator group receiving both clinician- and self-collected samples, which limits direct comparisons. The higher observed HPV positivity rate may have been influenced by the pre-assay heating protocol employed, though its clinical implications require further validation. Additionally, the time between kit receipt and sample collection could not be measured, as only the number of kits distributed and samples received were recorded. Hence, it remains possible that women outside the organised screening programme who submitted a self-sample were included in

the data, such as women who had been vaccinated against HPV and were subsequently offered an HPV self-sampling kit.

Conclusions

- The self-sampling approach detects a similar proportion of severe dysplasia as regular screening. Thus, our study indicated that self-sampling could replace primary HPV screening of cytology samples.
- The most powerful tool in the prevention of cervical cancer is attendance at the screening programme. Finding targeted improvements to increase attendance at the screening programme is vital for success in minimising cervical cancer in the future.
- Prolongation with HPV screening among elderly women may reduce the incidence of cervical cancer, which today is usually discovered when symptoms appear. Further quality assurance audits are necessary to continuously evaluate the effects of the primary HPV screening programme and to detect shortcomings requiring further assessment.
- Our results suggest that most women appreciated the self-test, although their perception of their capacity to perform it varied.
- HPV self-testing can help overcome the emotional and practical barriers to attending cervical cancer screening programmes.
- Anxiety relating to cervical dysplasia and a positive HPV test were reported; thus, healthcare professionals should provide tailored information regarding individual test results to help mitigate negative emotions concerning test results.
- Direct mailing of self-sampling kits effectively facilitated HPV detection and early intervention, demonstrating its potential for cervical cancer prevention despite limited initial adherence.

Future perspectives

Upcoming studies

Currently, I have two upcoming studies. Semi-structured in-depth interviews have been performed on 16 previously non-attenders who submitted the questionnaire in Paper III. This study aimed to further investigate their experiences in the screening programme and evaluate reasons for non-attendance. The other ongoing project is a retrospective cohort study with the primary aim of evaluating the cumulative incidence rate of CIN2+ in histopathology across different screening modalities (i.e. cytology, vaginal HPV self-sampling, and cervical HPV sampling). The secondary outcome is the incidence of invasive cervical cancer by screening modality among test-negative women (cytology or HPV). The study will be conducted first in the southern part of Sweden and thereafter nationally.

Will cervical cancer be eliminated?

Time has passed since the discovery that HPV infections cause cervical cancer, and the introduction of HPV vaccines on the market, yet cervical cancer remains a global health concern, and there is a need for rapid improvements and targeted interventions to accelerate the elimination of cervical cancer. In 2030, the WHO goal is to eliminate cervical cancer as a public health concern with an incidence below 4/100,000 cervical cancer cases yearly (21). Sweden has good conditions to make this a reality. As of 2023, 91% of all girls had received at least one dose of the HPV vaccine in grade 5, and 87.3% had received two doses of HPV-vaccination. A slightly lower coverage was observed among boys of the same age group, at 86.9% for one dose and 82.2% for two doses (189). Globally, however, by 2025 only 15 of the 132 WHO member states had achieved the WHO target of 90% vaccination coverage for the first dose (190). In Sweden, a “catch-up” vaccination with concomitant HPV testing for women born in the period 1994-1999 was introduced in Sweden in 2021, aiming for an even faster elimination of cervical cancer (23). In eight regions, more than 50% of all women born between 1994 and 1999 have received HPV vaccination in combination with HPV screening (105). The rationale behind the catch-up vaccination combined with concomitant HPV testing was to focus on the age groups in which HPV transmission primarily occurs, as these women are not fully vaccinated against HPV. Predictive

models using basic reproduction number (R_0) estimates have demonstrated that the R_0 value among women older than 35 falls below 1, indicating that HPV infections are unlikely to be maintained in women above 35 years if younger women cease to spread the virus to this age group (191). A significant decline in the prevalence of HPV16 and HPV18 has been observed in vaccinated birth cohorts compared to pre-vaccination cohorts. HPV16 and HPV18 prevalence declined by 98% and 99%, respectively, among the 2000 birth cohort relative to the 1984 cohort, with corresponding odds ratios of 0.02 (95% CI, 0.01–0.04) and 0.01 (95% CI, 0.00–0.04) (54). However, the prevalence of HPV types not covered by the vaccine has remained stable across different age groups (23). Following the introduction of the quadrivalent HPV vaccine, a study in the United States observed a substantial reduction in the prevalence of HPV types 6, 11, 16, and 18, with decreases of 71% among 14–19-year-old females and 61% among those aged 20–24, when compared to the pre-vaccine period (2003–2006). However, among females aged 25-34, there was no statistically significant decrease in the prevalence of the HPV types targeted by the quadrivalent vaccine. HPV types not covered by the quadrivalent vaccine did not seem to increase. A suggested herd immunity was also noted as declines in HPV prevalence targeted by the vaccine were also observed among the unvaccinated females (192).

To eliminate cervical cancer as a global health problem, WHO also states that 70% of women should have at least two HPV screening tests in their lifetime, and 90% of women with premalignant lesions or 90% with invasive cervical cancer should have received appropriate treatment. Across the entire age group of 23- to 70-year-old women, the average national screening coverage in 2023 was 77.7%. Coverage is defined as the proportion of women who have undergone a cervical screening test within the recommended interval, i.e. 5.5 years for women aged 23 to 50, and 7.5 years for those aged 51 to 70 (105). The majority of women (96.4%) with high-grade cervical lesions or cancer detected through cytology in 2022 underwent a biopsy within one year (193). In 2023, the overall cervical cancer incidence rate in Region Skåne was 9.68 per 100,000 women, compared to the national incidence rate of 11.64 per 100,000 women (153).

Cervical cancer screening coverage shows significant global disparities: 84% of women aged 30-49 in high-income countries have been screened at least once, while this figure drops to 48% in upper-middle-income countries, 9% in lower-middle-income countries, and 11% in low-income countries. Despite carrying the highest burden of disease, low- and middle-income countries exhibit markedly low implementation of HPV screening programmes (194). Insufficient infrastructure, limited access to appropriate equipment, health-workforce shortages, high rates of loss to follow-up, and sociocultural and informational barriers may all contribute to the challenges low-income countries face in implementing efficient HPV-based screening programmes. The WHO recommends screen-and-treat models to reduce attrition; in resource-limited settings, this often entails visual inspection with acetic acid followed by immediate treatment (21).

To screen or not to screen?

At what point is it appropriate to discontinue screening? Will other HPV types become more prevalent and contribute to new cases of cervical cancer as the most oncogenic high-risk types decline toward extinction? Additional considerations include population migration, which may introduce individuals with differing HPV prevalence and risk profiles for persistent infection.

Potential genotype replacement and clinical unmasking are two key phenomena of interest in the post-vaccination era. A shift in genotype prevalence may be observed when vaccine-targeted highly oncogenic HPV types decline, potentially enabling less oncogenic types to contribute more significantly to cervical disease. Clinical unmasking refers to the potential increase in cervical precancerous lesions caused by non-vaccine-targeted HPV types among women who have received HPV vaccination. It is proposed that among HPV-vaccinated women, fewer cervical treatments result in the preservation of the cervical transformation zone, where dysplasia associated with HPV infection commonly develops. Clinical unmasking was evaluated in a previous study conducted in Costa Rica with a follow-up period of up to 11 years. Included participants were aged 18-25 and were either randomised to receive the bivalent vaccine targeting HPV16 and 18, or to a control group. The results showed that vaccinated women had significantly higher rates of CIN2+ and CIN3+ lesions attributed to non-preventable HPV types compared with unvaccinated participants in the long-term follow-up. Furthermore, the authors argue that the full impact of clinical unmasking may not become apparent until a longer follow-up period has elapsed, particularly given the lower oncogenic potential of non-targeted HPV types (195). According to a meta-analysis, the introduction of the bivalent HPV vaccine has not been associated with a significant shift or replacement of HPV types (196). In two other studies the prevalence of HPV types not covered by the vaccine has remained stable across different age groups (23, 192).

Future screening programmes in the post-vaccination era

As the proportion of HPV-vaccinated women within the screening-eligible population increases, future screening programmes will require updated guidelines tailored to this demographic. In the interim, it may be necessary to differentiate screening protocols for vaccinated versus unvaccinated individuals. Without such adaptations, current screening programmes risk becoming suboptimal, potentially leading to increased healthcare costs and unnecessary interventions that may result in more harm than benefit. Several studies have advocated de-intensified screening programmes when fully HPV-vaccinated women in preadolescent ages enters the screening programme (197-199). A mathematical modelling study conducted in the

United States suggested that, among fully vaccinated females, cervical cancer screening should ideally begin at a later age, employ extended screening intervals, and utilise primary HPV testing to minimise potential harms associated with screening. For women vaccinated with the nonavalent HPV vaccine, the most efficient screening strategy was found to be primary HPV testing every ten years, starting at either age 30 or 35 (197). One study estimated the lifetime risk of developing CIN3+ among women vaccinated with the nonavalent HPV vaccine to be as low as 0.5% (198). Another study suggested that, for this population, two lifetime screening rounds may be sufficient to maintain effective cervical cancer prevention (199). One proposed approach to organising screening once HPV is no longer transmitted in the general population, as suggested by Dillner et al., is to implement a one-time screening to detect any remaining HPV-induced precursor lesions (191).

In a study by Matejka et al., the accuracy of HPV testing in vaccinated birth cohorts with the bivalent vaccine was discussed, addressing key factors that may influence the epidemiology of non-16/18 HPV genotypes. These factors included the protective immunity against HPV 16/18, potential cross-protection against other genotypes, and phenomena such as viral and clinical unmasking. An increase in HPV test specificity but a decrease in the PPV for CIN2+ might be observed. With fewer high-risk HPV infections circulating, fewer HPV-positive women without any precancerous lesions will be detected, while the PPV will decrease since non-HPV16/18 infections are less likely to cause high-grade lesions. Previously co-occurring HPV types (non-16/18) are detected more readily due to the phenomenon of viral unmasking. Consequently, viral unmasking could lead to more HPV-positive results that do not indicate disease, as these genotypes are less aggressive than HPV16/18, which might reduce the specificity and the PPV for CIN2+. In contrast, clinical unmasking might increase the specificity and PPV of HPV tests as more actual CIN2+ lesions from non-preventable HPV types come to light (200).

The high NPV of an HPV test allows the screening intervals to be safely extended. Since HPV testing results in more follow-up evaluations compared to cytology, costs can be reduced by extending the screening intervals. Additionally, further triage methods that improve specificity can help reduce the number of unnecessary gynaecological examinations and follow-up procedures (151).

In an editorial paper, discontinuation of cervical screening among HPV-vaccinated women was discussed. In response to widespread HPV vaccination, revised cervical screening protocols were suggested by the authors, incorporating a more refined balance of benefits and harms tailored to the vaccinated cohorts. Nevertheless, discontinuing cervical screening was argued to be inappropriate at this stage. One critical concern is the unvaccinated elderly population, and as the incidence of non-HPV16/18 cervical cancer also increases with age and accounts for approximately half of cervical cancer cases in older women, this group remains at risk. Vaccination with the bivalent and quadrivalent vaccines does not protect against the remaining

oncogenic types responsible for around 30% of cervical cancers. Additionally, individuals vaccinated with the nonavalent vaccine from 2019 will not reach the recommended screening age for several years (201).

Extending the upper age limit for cervical cancer screening could be considered in light of increasing life expectancy. In our audit (Paper II), a substantial proportion of women were diagnosed with cervical cancer after exiting the screening programme. Older women also present specific challenges, as cytology testing demonstrates reduced sensitivity in this subgroup, and practical difficulties related to biopsy sampling and colposcopic evaluation are more pronounced due to age-related hormonal changes with the transformation zone higher up and invisible in the cervix (TZ3).

HPV genotypes in future screening

In the post-vaccination era, it has been proposed that screening for hrHPV types should be limited to the seven hrHPV types covered by the nonavalent vaccine to improve the efficiency of screening programmes (89). Prioritising the detection of HPV types with the highest oncogenic potential allows for a more effective allocation of healthcare resources, ensuring that efforts are concentrated where they yield the most significant public health benefits. The inclusion of HPV genotypes associated with only a small percentage of invasive cervical cancer decreases the predictive value of screening, thereby reducing its efficiency and cost-effectiveness. This targeted approach enhances the effectiveness of screening programmes while minimising the risks of overdiagnosis and unnecessary follow-up procedures (90). A 2024 systematic review estimated the global prevalence of different HPV genotypes in invasive cervical tumours, and identified 17 distinct genotypes (202). However, four of these HPV types (namely 73, 26, 69 and 82) are classified as possibly carcinogenic (group 2B) by the IARC (42). Their prevalence was low, with HPV 73 detected in 0.7% of cases and the remaining three genotypes each accounting for approximately 0.3%. HPV16 was the most prevalent genotype, accounting for 61.7% of cases, with an additional 20.1% attributed to HPV18 (15.3%) and HPV45 (4.8%). Eight HPV genotypes demonstrated the highest attributable fractions and were considered the most carcinogenic: 16, 18, 31, 33, 35, 45, 52, 58. The nonavalent vaccine provides coverage for all these HPV genotypes except for HPV35, which was found to be more prevalent in Africa (attributable fraction 3.6), compared to other regions (0.6-1.6%) (202).

Promising triage methods for the future

Given the high prevalence of HPV in vaginal self-samples, which contributes to high referral rates, it is important to have more specific triage tests to separate transient HPV infections from progressive HPV infections that will cause cervical intraepithelial neoplasia and potentially cervical cancer. A repeat HPV test four to six months after a positive result can help distinguish transient infections from those more likely to persist (145). Promising triage markers for the future could include DNA methylation (35), microRNA expression (149), or p16/Ki-67 dual staining (36). Next-generation sequencing presents valuable applications for improving quality assurance in HPV screening, as well as for monitoring the landscape of HPV variants and assessing the potential emergence of novel variants in the post-vaccination era (203). Furthermore, a higher viral load has been associated with a greater risk for HPV persistence, and can be quantified concurrently with HPV detection using droplet digital PCR (204). Lastly, in resource-limited settings, a more cost-efficient method with rapid HPV detection using isothermal amplification is a promising alternative for cervical cancer screening (205).

Final thoughts

While triage algorithms and flowcharts can support initial clinical decision-making, individualised assessment remains essential, requiring a comprehensive evaluation of risks and benefits for each patient. In consultations at the colposcopy clinic, it is crucial to conduct a personalised risk assessment for each woman. Women with a history of cervical dysplasia are at increased risk of developing future cervical intraepithelial neoplasia (102). Additional risk factors for HPV persistence, including co-infection with other sexually transmitted infections (such as *Chlamydia trachomatis*, herpes simplex virus type 2, and HIV), immunosuppression (10), early sexual debut, multiple sexual partners (58), a higher number of full-term pregnancies (206), smoking, and long-term use of oral contraceptives (57), should also be taken into account. Furthermore, decisions regarding follow-up procedures are also influenced by factors such as age, reproductive history (including previous or planned pregnancies), sexual behaviour, and the duration of HPV persistence. Finally, psychological aspects must not be overlooked; many women experience significant anxiety following referral to a colposcopy clinic. Thus, providing empathetic care, emotional support, and individualised information is a key component of patient-centred management.

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