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Benefits and harms of HPV screening

Maija Vahteristo

DOCTORAL DISSERTATION

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University of Helsinki

Supervisors

Docent
Sirpa Heinävaara
University of Helsinki

Docent
Maarit Leinonen
University of Helsinki

Reviewers

Docent
Annika Auranen
University of Turku

Professor
Karolina Louvanto
University of Tampere

Official opponent

Miriam Elfström
Karolinska Institutet

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Abstract

In Finland, the population-based cytology screening programme has successfully reduced the incidence and mortality of cervical cancer by over 80% since its initiation in 1963. In recent years, HPV screening has replaced traditional cytology screening in many high-income countries, including Finland. A pooled analysis of European randomised trials with moderate follow-up duration showed that HPV screening offers greater protection against cervical cancer than cytology screening. In this thesis, we estimated the long-term effects of HPV screening on cervical cancer incidence and mortality in a setting with a long-standing and highly effective cytology screening programme. Additionally, we assessed the effectiveness of cervical HPV screening on the incidence and mortality of other HPV-related anogenital cancers, a previously unexplored area.

While HPV screening offers greater sensitivity, it also introduces harms compared to cytology screening. Without an effective triage strategy for HPV-positive individuals, the lower specificity of HPV screening results in higher colposcopy referral rates and a potential overdiagnosis of non-progressive cervical lesions. This thesis aimed to identify an optimal HPV screening algorithm using cytology as a triage method for HPV-positive individuals. Furthermore, we evaluated the utility of extended HPV genotyping in enhancing risk stratification within the screening programme.

All of the analyses in this thesis were based on the long-term follow-up data from the Finnish randomised HPV screening trial, which took place from 2003 to 2013. This included individual-level register data from four different population-based health registries, as well as HPV genotyping results for 5253 HPV screening samples from the trial's first screening round.

Over up to 18 years of follow-up, HPV screening demonstrated similar effectiveness to cytology screening in reducing cervical cancer incidence and mortality. However, vaginal cancer incidence was 60% lower in the HPV screening arm compared to the cytology arm, and a non-significant reduction was also observed for vulvar cancer incidence. Although HPV screening did not demonstrate improved effectiveness for cervical cancer prevention in the context of Finland's well-established cytology programme and low baseline risk, it is likely to offer greater protection in other settings. Our findings also suggest a potential novel

benefit of HPV screening in preventing other HPV-related anogenital cancers. While this observation requires further validation, it indicates that individuals at high risk of vaginal and vulvar cancers may also benefit from HPV screening.

Our evaluation of HPV screening algorithms with cytology triage found the best balance between sensitivity and specificity for detecting cervical precancer and cancer by managing HPV-positive, cytology-negative individuals with two retests. However, all HPV screening algorithms with cytology triage resulted in higher colposcopy referral rates compared to cytology screening alone. Extended HPV genotyping, on the other hand, showed promising potential for risk stratification, particularly when combined with cytology triage results. For most genotypes, cytology provided additional risk stratification information. High-risk genotypes such as HPV16, 31, and 33/58 were associated with elevated risk even in the presence of normal cytology. Conversely, HPV51 and HPV56/59/66 showed low associated risk even when cytological abnormalities were present.

Cytology triage alone is an insufficient method to reduce the increased colposcopy referral rates associated with HPV screening. However, when extended HPV genotyping is incorporated into screening algorithms, cytology triage can provide additional risk stratification information. Including extended genotyping in screening strategies may help reduce unnecessary colposcopy referrals.

Tiivistelmä

Suomessa 1963 käynnistynyt väestöpohjainen kohdunkaulasyövän seulontaohjelma on onnistunut vähentämään kohdunkaulasyövän ilmaantuvuutta ja kuolleisuutta yli 80 %. Viime vuosina HPV-testit ovat korvanneet sytologiset testit monessa korkean tulotason maassa, mukaan lukien Suomessa. Meta-analyysi satunnaistetuista tutkimuksista osoitti että HPV-seulonta tarjoaa paremman suojan kohdunkaulasyöpää vastaan kuin sytologinen seulonta. Tässä väitöskirjassa arvioin HPV-seulonnan pitkäaikaisvaikutuksia kohdunkaulasyövän ilmaantuvuuteen ja kuolleisuuteen Suomessa, ympäristössä, jossa pitkään jatkunut sytologinen seulonta on ollut hyvin vaikuttavaa. Lisäksi arvioin HPV-seulonnan vaikuttavuutta myös muiden HPV-välitteisten anogenitaalialueen syöpien ilmaantuvuuteen ja kuolleisuuteen, joka on aihealue, jota ei ole aiemmin tutkittu.

Vaikka HPV-testi on herkempi kuin sytologinen testi, HPV-seulontaan liittyy myös haittoja. Ilman sopivaa jatkotutkimustestiä HPV-positiivisille henkilöille, seulonnan alhaisempi tarkkuus johtaa suurempaan määrään jatkotutkimuslähetteitä ja mahdolliseen ei-etenevien kohdunkaulasyövän esiasteiden ylidiagnostiikkaan. Tämän väitöskirjan toisena tavoitteena oli etsiä jatkotutkimusmenetelmiä, jotka vähentävät HPV-seulonnan haittoja. Etsin parasta tapaa hyödyntää sytologista testiä seulonnassa HPV-positiivisten jatkotutkimuksena, sekä arvioin laajennetun HPV-genotyypityksen hyödyllisyyttä seulontaohjelmassa.

Kaikki tämän väitöskirjan osajulkaisut perustuvat satunnaistetun, vuosina 2003–2013 käynnissä olleen HPV-seulontatutkimuksen pitkäaikaisseurantaan. Tässä väitöskirjassa muodostettu pitkäaikaisseuranta-aineisto sisälsi yksilötason rekisteritietoja neljästä väestöpohjaisesta terveysrekisteristä, sekä laajennetun HPV-genotyypityksen tulokset yhteensä 5253:sta HPV-näytteestä.

18 vuoden maksimiseurannan aikana HPV-seulonta ei johtanut parempaan vaikuttavuuteen kohdunkaulasyövän ilmaantuvuuteen tai kuolleisuuteen kuin sytologinen seulonta. Sen sijaan emättimen syövän ilmaantuvuus oli 60 % alhaisempi HPV-seulontaryhmässä verrattuna ryhmään, jota seulottiin pelkällä sytologisella testillä. Pienempi, ei-merkitsevä, ero havaittiin myös ulkosynnyttimien syöpien ilmaantuvuudessa. Vaikka HPV-seulonta ei osoittanut parempaa vaikuttavuutta kohdunkaulasyövän ehkäisyssä Suomen olosuhteissa, joissa kohdunkaulasyövän ilmaantuvuus on hyvin matala seulontaan osallistuvien

keskuudessa, se on todennäköisesti sytologiaseulontaa vaikuttavampi muissa olosuhteissa. Väitöskirjan tulokset viittaavat myös siihen, että HPV-seulonta ehkäisee HPV-välitteisiä vaginan ja vulvan syöpiä.

Paras herkkyyden ja tarkkuuden tasapaino hyödyntämällä sytologista testiä HPV-positiivisten jatkotutkimuksena, saavutettiin seulonta-algoritmilla, jossa HPV-positiiviset henkilöt, joilla ei ollut sytologisia poikkeamia kutsuttiin kahteen riskiryhmätestiin ennen kolposkopiaan lähettämistä. Kaikki seulonta-algoritmit, joissa käytettiin pelkkää sytologista testiä HPV-positiivisten jatkotutkimuksena, johtivat kuitenkin suurempaan määrään kolposkopialähetteitä verrattuna perinteiseen sytologiseen seulontaan. Laajennetun HPV-genotyyppitiedon hyödyntäminen seulonnassa sen sijaan on lupaava jatkotutkimusmenetelmä, sekä vähentämään lähetteitä, että ennustamaan esiaste- ja syöpäriskiä. Useimpien genotyyppien kohdalla lisäämällä sytologinen testi genotyyppitietoon, paransi riskiluokitusten tarkkuutta. Korkeimman riskin genotyyppien, HPV16, 31 ja 33/58, infektiot johtivat kohonneeseen riskiin myös silloin kun sytologia oli normaali. Sen sijaan tyypeillä HPV51 ja HPV56/59/66 oli matala riski, vaikka sytologia oli poikkeava.

Pelkkä sytologinen testi HPV-positiivisten jatkotutkimuksena ei siis riitä vähentämään HPV-seulonnan kolposkopiakuormaa. Kun seulonta-algoritmeihin sisällytetään tieto HPV-genotyypistä, voi sytologinen testi tarjota lisäarvoa riskiluokituksessa. HPV-positiivisten laajennettu genotyypitys on lupaava jatkotutkimusmenetelmä vähentämään tarpeettomia kolposkopioita.

Abbreviations

95% CI	95% confidence interval
AF	Attributable fraction
AGCNOS	Atypical glandular cells not otherwise specified
ASCC	Anal squamous cell carcinoma
ASC-H	Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion
ASCUS	Atypical squamous cells of undetermined significance
ASR (W)	Age-standardised rate (world standard population)
CIN1	Cervical Intraepithelial Neoplasia Grade 1
CIN2	Cervical Intraepithelial Neoplasia Grade 2
CIN3	Cervical intraepithelial neoplasia grade 3
CIN2+	Cervical intraepithelial neoplasia grade 2 or more severe
CIN3+	Cervical intraepithelial neoplasia grade 3 or more severe
DNA	Deoxyribonucleic acid
E6	Early region oncoprotein E6
E7	Early region oncoprotein E7
FCR	Finnish Cancer Registry
HC2	Hybrid Capture 2 (HPV test)
HSIL	High-grade squamous intraepithelial lesion
HSIL+	High-grade squamous intraepithelial lesion or worse
HPV	Human papillomavirus
IARC	International Agency for Research on Cancer

ICD-O-3	International Classification of Diseases for Oncology, Third Edition
IRR	Incidence rate ratio
ITT	Intention-to-treat (principle in the analysis of trials)
Ki-67	A protein expressed during active phases of the cell cycle, overexpressed during HPV-induced carcinogenesis
L1	Major late capsid protein L1
LBC	Liquid-based cytology
LSIL	Low-grade squamous intraepithelial lesion
LSIL+	Low-grade squamous intraepithelial lesion or worse
MRR	Mortality rate ratio
NILM	Negative for intraepithelial lesion or malignancy
NPV	Negative predictive value
Pap test	Papanicolaou test
PCR	Polymerase chain reaction
PPV	Positive predictive value
p16	Also known as $p16^{INK4a}$, a tumour suppressor protein overexpressed in HPV-induced carcinogenesis
p53	Tumour protein p53
pRB	Retinoblastoma protein
RCT	Randomised controlled trial
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
TBS	The Bethesda system
VaIN1	Vaginal intraepithelial neoplasia grade 1
VaIN2	Vaginal intraepithelial neoplasia grade 2
VaIN3	Vaginal intraepithelial neoplasia grade 3
VIN1	Vulvar intraepithelial neoplasia grade 1

VIN2	Vulvar intraepithelial neoplasia grade 2
VIN3	Vulvar intraepithelial neoplasia grade 3
WHO	World Health Organization

Glossary

Algorithm characteristics	The sensitivity, specificity, and colposcopy referral rates of the studied post-hoc algorithms.
Colposcopy	A diagnostic procedure in which the cervix, vagina, and vulva are examined using a magnifying instrument. If suspicious findings are observed, a biopsy may be taken during the procedure to confirm the diagnosis. If a high-grade cervical lesion is detected, a loop electrosurgical excision procedure may be performed to treat the lesion.
Cumulative incidence	Cumulative incidence describes all new cases of a given outcome occurring within a specified time interval. Can be expressed as the proportion of the studied population who develop the outcome during that period.
DNA methylation	A triage method in cervical cancer screening that detects epigenetic changes associated with carcinogenesis in human and papillomavirus genes.
Effectiveness of cervical cancer screening	How well cervical cancer screening reduces the incidence and mortality of cervical cancer.

Extended HPV genotyping	Detection of a broader range of carcinogenic HPV genotypes or type groups, beyond HPV16 and HPV18.
Full HPV genotyping	Individually detects all carcinogenic HPVs genotypes.
Incidence	Rate of new cases over a specified period for the population at risk.
Intention to treat analysis	A method in the analysis of trials, where all individuals are analysed in the groups to which they were originally randomised, regardless of whether they completed the intervention as planned.
Mortality	Rate of deaths over a specified period for the population at risk.
p16/Ki-67 dual staining	A triage method in cervical cancer screening that detects the simultaneous expression of p16 and Ki-67 proteins in cervical cells.
Partial HPV genotyping	Reporting only HPV16 and HPV18 individually, and other types pooled.
Per protocol analysis	A method in the analysis of trials, only individuals who adhered to the study protocol are included in the analysis.
Primary screening test	The initial test used in the screening programme.
Prevalence	The proportion of individuals in a population who have a specific disease or condition at a given point in time.
Rate ratio	A measure comparing the incidence or mortality rates of an event between two groups over a specified period.

Risk stratification	In screening, categorising individuals into different groups with tailored management strategies based on their risk of having or developing the screened disease.
Screening algorithm	A pathway consisting of the primary screening test, triage test(s), decisions made based on the test results and possible follow-up tests. Does not include possible diagnostic follow-up procedures.
Sensitivity of a test	Probability of a positive test result given that the individual has the condition tested for.
Specificity of a test	Probability of a negative test result given that the individual does not have the condition tested for.
Triage	A secondary test performed for those who have tested positive in the primary screening test.

Index

List of original publications

This thesis is based on the following publications:

I Vahteristo, M., Leinonen, M.K., Sarkeala, T., Anttila, A., Heinävaara, S., 2024. Similar effectiveness with primary HPV and cytology screening - Long-term follow-up of randomized cervical cancer screening trial. Gynecologic Oncology 180, 146–151.

II Vahteristo, M., Leinonen, M.K., Sarkeala, T., Anttila, A., Heinävaara, S., 2024. Lower incidence of vaginal cancer after cervical human papillomavirus screening - long-term follow-up of Finnish randomized screening trial. Preventive Medicine 185, 108031.

III Vahteristo, M., Heinävaara, S., Anttila, A., Sarkeala, T., 2022. Alternative cytology triage strategies for primary HPV screening. Gynecologic Oncology 167, 73–80.

IV Vahteristo, M., Heinävaara, S., Sarkeala, T., Dillner, J., Kalliala, I., Nieminen, P., Leinonen, M.K.,. Long-term human papillomavirus genotype-specific risk of cervical high-grade intraepithelial lesion and cancer—By age group and triage cytology. International Journal of Cancer. 2025;1-11.

The publications are referred to in the text by their roman numerals.

1 Introduction

Human papillomavirus (HPV) causes 4.5% of all cancers, and 8.3% of all cancers in women worldwide. [1] HPV vaccination is expected to reduce the incidence of HPV-related malignancies in the countries with high vaccination coverage in the coming decades. However, as the youngest vaccinated cohorts are only entering cervical screening programmes and vaccination coverage remains suboptimal in many regions [2], the importance of effective cervical cancer screening has not diminished. At the same time, cervical cancer screening is undergoing its biggest transformation to date, as HPV testing is replacing traditional cytology as the primary screening method. HPV screening has been implemented in numerous countries, particularly among high-income nations [3], and is currently recommended as the primary screening method by the European Guidelines for Quality Assurance in Cervical Cancer Screening. [4]

The effectiveness of HPV screening has been evaluated in randomised controlled trials, which demonstrated earlier detection of cervical precancers compared to cytology screening. [5–8] A pooled analysis of these trials showed that HPV screening offered greater protection against cervical cancer than cytology screening over a median follow-up of 6.5 years. [9] However, further data, particularly on the long-term effectiveness of HPV screening in reducing cervical cancer incidence and mortality are still warranted.

In addition to cervical cancer HPV is attributed to other anogenital cancers and oropharyngeal cancers. Other anogenital cancers are more rare than cervical cancer, but the incidence of some of them has been on the rise among younger individuals in high-resource countries. [10–12] The effectiveness of cervical HPV screening on other anogenital HPV-related cancers has not previously been assessed.

The specificity of HPV screening is, however, lower than that of cytology screening [13], resulting in higher colposcopy referral rates. Unnecessary colposcopies increase the harms associated with screening by placing a burden on both healthcare systems and the individuals being screened. The most effective way to address the lower specificity of HPV screening is through the use of an appropriate triage test for HPV-positive individuals. Currently, the recommended triage method for HPV-positive individuals is cytology. [4] However, countries that have implemented primary HPV screening with cytology triage have also reported

substantially higher colposcopy referral rates compared to cytology screening. [14–16] The specific HPV screening algorithms incorporating cytology triage differ across screening programmes, and the optimal management strategy, particularly for HPV-positive, cytology-negative individuals, has yet to be established.

Genotyping is currently the most promising option for the risk-stratification among HPV-positive individuals. Different HPV genotypes possess different risks for cervical precancers and cancers [17–19] and incorporating this information into the screening programmes is extremely useful. However, the long-term risks these genotypes possess in a screening programme, especially stratified with the baseline cytology triage result, have not been assessed.

The aims of this thesis were to evaluate the benefits of HPV screening by examining its effectiveness in reducing cervical cancer incidence and mortality, as well as the incidence and mortality of other HPV-related anogenital cancers. Furthermore, the thesis aimed to evaluate the potential of risk-stratified screening strategies incorporating cytology triage and HPV genotyping, with the goal of maximising both the specificity and sensitivity of screening. The research is based on long-term follow-up data from the Finnish randomised controlled HPV screening trial.

2 Review of the literature

2.1 HPV

Human papillomaviruses (HPVs) belong to the larger *Papillomaviridae* family, and are small circular double-stranded DNA viruses. The family can be classified into five genera: Alpha, Beta, Gamma, Mu, and Nu. [20] Of these, *Alphapapillomaviridae* viruses are the ones most associated with varying clinical conditions in humans, for example genital warts, benign lesions, and cancers. The HPVs in the other four genera mainly infect cutaneous epithelia. [21]

All HPV genomes consist of 3 regions: an upstream regulatory region, controlling viral transcription and replication, an early region, coding for proteins crucial in the carcinogenesis, and a late region, coding for the virion capsid proteins. The difference in carcinogenic potential arises specifically from the genetic differences in the genome early region. [22] E6 and E7, located in the early region, are the main HPV oncoproteins. Both of them target multiple human cell proteins, for example the known tumour suppressors p53 and retinoblastoma tumour suppressor protein (pRB). [23–25]

2.1.1.1 HPV genotypes

The above-mentioned HPV genera are further divided into species, and the *Alphapapillomaviridae* genus has a total of 14 species. Of these species, Alpha-5, Alpha-6, Alpha-7, Alpha-9, and Alpha-11 contain carcinogenic HPV genotypes. International Agency for Research on Cancer (IARC) has categorised 12 HPV genotypes from this genus as carcinogenic, 1 genotype as probably carcinogenic, and 11 genotypes as possibly carcinogenic to humans. [26] These genotypes can cause cancerous lesions in multiple anatomical sites, including cervix[27], oropharynx and other upper respiratory tract areas[28], penis[29], vagina, vulva, and anus [30]. The genotypes and their respective species are shown in Table 1.

Table 1 Carcinogenic, probably carcinogenic, and possibly carcinogenic HPV genotypes from the Alphapapillomavirus genus and their species.

The classification to carcinogenic, probably carcinogenic, and possibly carcinogenic is based on the IARC Human carcinogens—Part B: biological agents -monograph. [26]

Genotype	Species	IARC Classification
HPV 16	Alpha-9	
HPV 18	Alpha-7	
HPV 31	Alpha-9	
HPV 33	Alpha-9	
HPV 35	Alpha-9	
HPV 39	Alpha-7	
HPV 45	Alpha-7	Group 1: Carcinogenic to humans
HPV 51	Alpha-5	
HPV 52	Alpha-9	
HPV 56	Alpha-6	
HPV 58	Alpha-9	
HPV 59	Alpha-7	
HPV 68	Alpha-7	Group 2A: Probably carcinogenic to humans
HPV 26	Alpha-5	
HPV 30	Alpha-6	
HPV 34	Alpha-11	
HPV 53	Alpha-6	
HPV 66	Alpha-6	Group 2B: Possibly carcinogenic to humans
HPV 67	Alpha-9	
HPV 69	Alpha-5	
HPV 70	Alpha-7	
HPV 73	Alpha-11	
HPV 82	Alpha-3	

Most of these oncogenic genotypes belong to the Alpha-9 species: HPV16, HPV31, HPV33, HPV35, HPV52, and HPV58. Clearly the most carcinogenic is HPV16, a genotype known to be the main etiological agent behind squamous cell carcinomas of many different anatomical sites. [1] Alpha-7 genotypes, especially HPV18, have been linked with adenocarcinomas. [31–34] HPV51 is the only genotype from Alpha-5 species classified as carcinogenic to humans.

To be classified as a new distinct HPV genotype, the L1 region, located in the late genome region, of the new virus isolate must differ at least 10% of the L1 region of any known HPV type. [20] As double-stranded DNA viruses HPVs are genomically stable, and do not easily mutate into new types. Research has, however, shown the existence of different genetic variants of the most studied genotypes. [35,36] The carcinogenicity of these variants seems to also differ. [37] For example HPV16

comprises several genetic lineages, with varying carcinogenic potentials, also influenced by host-related factors. [38]

2.1.1.2 HPV-mediated carcinogenesis

HPV is a sexually transmitted virus, and HPV infection is the most common sexually transmitted infection in women. [39] It can be transmitted via vaginal or anal intercourse, and more rarely via oral sex. [40–43] Perinatal transmissions have also been detected, but they are rare and unlikely to result in a persistent infection. [44–46]

The persistence of HPV infection is a strong predictor of progression to malignancy. [47,48] Whether the infection persists or clinically clears (and either enters a latent state or not), depends on multiple factors. Known virus-related factors affecting the likelihood of persistence are HPV genotype [49], occurrence of multiple simultaneous infections [50], and viral load [51,52]. Host-related factors affecting HPV persistence include age [53], immunosuppression [54], and oral contraceptive use [55]. Other host-related factors, like parity, smoking, history of HPV-related malignancies, and genetic factors also affect individual's cervical cancer risk via different mechanisms. [56,57]

HPV-mediated carcinogenesis is a complex multistep process, also able to regress back to its earlier stages before it reaches the stage of invasion. HPV infections are initiated in the differentiating epithelium. [58] In the beginning of the infection, HPV genome is maintained in low copy numbers, not yet integrated to the host cell genome, in the undifferentiated cells of the basal epithelium. HPV genome is extremely reliant on the host cell replication and maintenance machinery. As the cells of basal epithelium containing the HPV genome differentiate and move up in the epithelial layer, HPV oncoproteins E6 and E7 disrupt the normal cellular processes to maintain the cellular replication of the differentiated cells. These disruptions largely occur through the inactivation of tumour suppressors, p53 and pRb. [59] In the later stages of the malignant transformation, HPV genome can integrate to the host cell genome, which accelerates the carcinogenesis. [60,61]

The genotype-specific mechanisms for carcinogenesis have mainly been researched on HPV16 and HPV18, and the mechanisms of other genotypes are still largely unresearched. Recent evidence has shown some differences in the HPV18-mediated carcinogenesis compared to the HPV16-mediated. Unlike in HPV16-infected stem-like cells, HPV18 genome integration occurs in early stages of the infection and the carcinogenesis may not be mediated through the differentiation of stratified squamous epithelium. Instead, the integrated HPV18 genome creates a latent persistent infection, difficult to be detected with the currently used methods. This could be one of the reasons behind the low HPV18 detection rate in precancerous lesions. [62] Another reason is that HPV18 is more associated with

adenocarcinoma, which does not have as easily detectable precancerous stages as squamous cell carcinoma. [31]

2.1.2 Cervical HPV infections

Most cervical HPV infections are asymptomatic and clear spontaneously through an immune response within 24 months. [63,64] There are also genotype-specific differences in the clearance and persistence patterns of HPV infections. The Finnish Family HPV Study showed that cervical HPV16 infections are the most persistent and least likely to clear, followed by infections with multiple HPV types. [65,66]

2.1.2.1 Cervical HPV prevalence

A meta-analysis of over one million women with normal cytology estimated the global prevalence of cervical HPV infection with any genotype to be 11.7%. [67] The prevalence estimates differ between geographical regions, being the highest in Sub-Saharan Africa at 24.0%, Eastern Europe at 21.4%, and Latin America at 16.1%. In Northern Europe, the estimated cervical HPV prevalence was 10.0%, which was near the global average. [67]

In addition to the differences in the overall prevalence estimates, also HPV genotype proportions differ geographically. HPV16 is the most prevalent in the areas where the overall HPV prevalence is the lowest. HPV18 is the second most prevalent type in all other regions except for Europe and Africa. Also notable is that in Europe the prevalence of HPV31 is a lot higher than in any other region. A Finnish study assessing genotype-specific prevalences, found out that the most prevalent genotypes in women with normal cytology were HPV16, followed by HPV31, and HPV52. HPV18 was only the 7th most prevalent type among Finnish population in this analysis. [68]

The age-specific cervical HPV prevalence curve follows either a unimodal or a bimodal pattern, depending on the geographical area. [69,70] The first peak occurs in women below 25 years old. The possible second peak is the most pronounced in some parts of Africa, and America, and occurs in women older than 45. [70–72] Based on research, these second-peak infections are more likely to be caused by reactivations of latent HPV infections than by new infections. [73]

Papillomaviruses seem to be able to stay latent in the basal cells of epithelia, with low DNA copy numbers and minimal gene expression even when HPV is not detected. [74] During the last decade research has provided a growing body of evidence on the papillomavirus latency in humans; genotype-specific re-detection or reactivation can occur in individuals who have cleared their previous HPV infections. [75–77] Thus an updated model for the natural history of cervical HPV infection has recently been published, also incorporating viral latency [78], Figure 1.

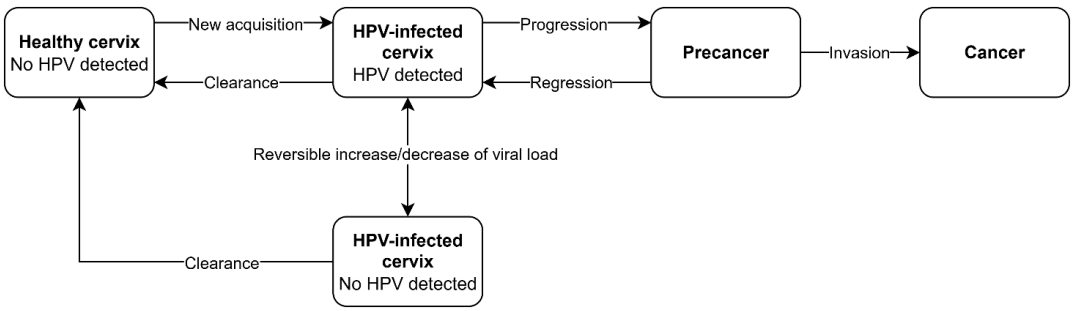


Figure 1 Natural history of cervical HPV infection. Modified from Lycke et al. 2025. [78]

2.1.2.2 Cervical precancers

The risk of cervical precancer is highest among individuals who have a persistent HPV infection with the same genotype. [18,79] Cervical precancers can be categorised into cervical intraepithelial neoplasia grade (CIN) 1, 2, and 3, based on the extent of abnormal cell growth within the cervical epithelial layer. CIN1 is a mild dysplasia, visible only in the lower third of cervical lining. CIN2 is a moderate dysplasia, expanding to the middle third of cervical lining. CIN3 is a severe dysplasia, expanding to more than two-thirds of cervical lining. [80]

The spontaneous regression rates of conservatively managed histological CIN1 and CIN2 lesions are similar, 60% and 55%, respectively. Of CIN1s 11% progress to CIN2 or more severe lesions (CIN2+) and 2% to CIN3 or more severe lesions (CIN3+). Of CIN2s 19% progress to CIN3+. The estimates for CIN3 regression and progression under conservative management are much more uncertain, as it is not ethical to leave CIN3 cases untreated. However, the estimated regression rate is around 28% and progression rate to cancer 2%. [81]

Even though the HPV prevalence and genotype distribution in CINs differ from those in cervical cancers, HPV16 is also the most common genotype among all three CIN categories. [82–84] The estimated prevalence of any high-risk HPV is the highest among CIN3s and the lowest among CIN1s. [82,83,85] Among CIN1s HPV types classified as non-carcinogenic are more common. [86] This suggests that the aetiology of CIN1 is different to the more severe lesions, and that it is not a needed predecessor of the more severe lesions. A Finnish study on age-specific genotype distributions among CIN2+ cases, showed that HPV16 and HPV18 were more common among lesions of individuals under 30 years old, and the other high-risk genotypes were more common among older individuals. [87]

In 2012, a two-tiered classification for cervical precancers was recommended. [88] As the histologic grading of especially CIN2 lesions has been shown to have a high interobserver variability and low reproducibility [89], CIN2s and CIN3s are both classified as high-grade intraepithelial lesions (HSIL) in the two-tiered system and CIN1s are classified as low-grade intraepithelial lesions (LSIL). The two-tiered classification also created a unified system for HPV-related lesions across lower anogenital sites, as cervix is not the only anogenital site with HPV-related malignancies. [90] Table 2 shows the correspondence between the old and the new classification systems.

Table 2 Classification of cervical lesions

In 1968 Richart proposed the CIN classification system of cervical lesions, shown on the left column. In 2012 a new two-tiered classification system based on the Bethesda System for cervical lesions was recommended, shown in the middle column.

Richart classification	**The Bethesda System**	**Description**
CIN (cervical intraepithelial neoplasia) 1	LSIL (low-grade squamous intraepithelial lesion)	Mild dysplasia, visible in the lower third of cervical lining
CIN2	HSIL (high-grade squamous intraepithelial lesion)	Moderate dysplasia, expanding to the middle third of cervical lining
CIN3	HSIL	Severe dysplasia, expanding to more than two-thirds of cervical lining

2.1.3 Cervical cancer

The first link between HPV and malignancy was established in cervical cancer. Since the 1970s, a large amount of experimental and epidemiological evidence has shown a clear association between the HPV infection and cervical carcinogenesis. [91,92] Nowadays, HPV is considered to be the necessary cause of almost all cervical cancers. [93]

2.1.3.1 Cervical cancer incidence and mortality

In 2022, there were more than 660 000 new cervical cancer cases worldwide, and almost 350 000 cervical cancer deaths. The incidence and mortality of cervical cancer vary significantly across geographic regions and are strongly influenced by socioeconomic factors, being lower in high-income countries due to effective long-lasting screening programmes and higher treatment resources. In countries with a high or very high human development index, the age-standardised cervical cancer incidence is on average 12.1 per 100 000, and mortality 4.8 per 100 000. In countries with a low or medium human development index the incidence is 19.3 per 100 000, and mortality 12.4 per 100 000. [94] These numbers remain well above the threshold set by the Global Cervical Cancer Elimination Initiative by World Health Organization (WHO), which is less than 4 cases per 100 000 women per year. [95]

Finland is one of the countries with the lowest incidence and mortality rates of cervical cancer, age-standardised incidence at 5.4 per 100 000 and mortality at 1.0 per 100 000 in 2023. [96] The incidence of cervical cancer drastically dropped after the initiation of cervical cancer screening in 1963 and has stayed on a low level ever since, Figure 2. By the 1990s, also an 80% decrease in the cervical cancer mortality was observable. [97]

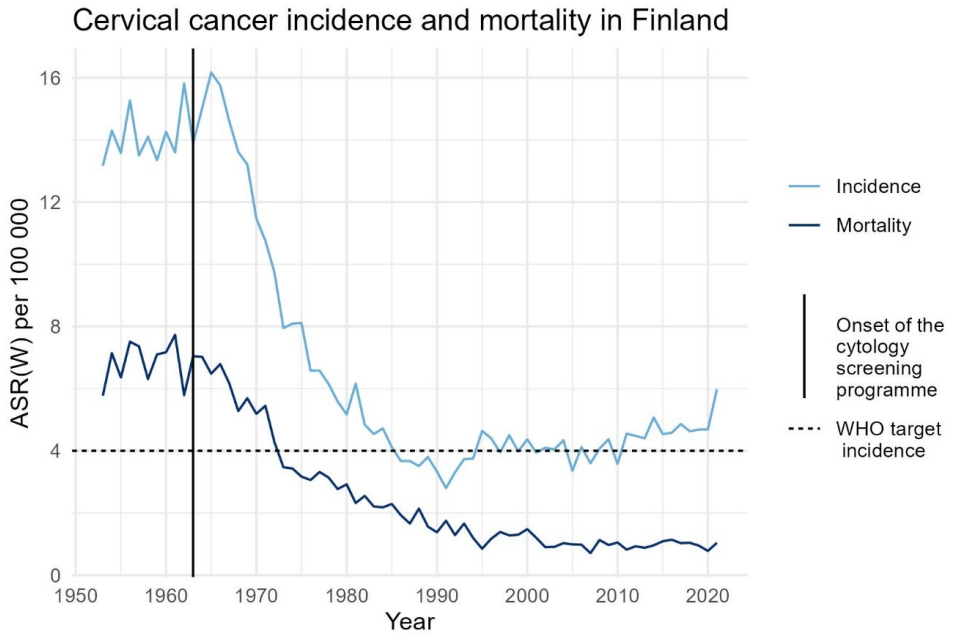


Figure 2 Cervical cancer incidence and mortality throughout the decades in Finland. The y-axis represents age-standardised (to the world population) (ASR (W)) rates per 100 000 individuals. Incidence curve is shown in light blue, and mortality curve in dark blue. The black vertical line represents the onset year of the cytological screening program, 1963. The dashed line represents the target cervical cancer incidence set by World Health Organization (WHO), 4 per 100 000.

In recent decades, a slight increase in cervical cancer incidence has been observed in Finland and other high-income countries, particularly among women aged 30–39. [97–99] Possible suggested reasons include increased HPV prevalence, reduced screening coverage among younger age groups, and changes in screening programmes, like prolonged screening intervals and insufficient quality of cytological screening. [98,100,101] However, the cervical cancer mortality rates have either remained stable or decreased in these countries, probably reflecting successful early detection via screening, and the high quality of cervical cancer treatment. [98] In the Nordic countries, the relative five-year survival for cervical cancer has remained relatively stable, with a slight improvement observed over recent decades. [102]

In Finland there are also large demographic differences in cervical cancer incidence and mortality. First-generation immigrants from Non-Western countries have a significantly higher cervical cancer incidence and mortality than non-immigrant women. [103] The risk is greatest among those with the shortest duration of residence and the oldest age at immigration, highlighting the influence of HPV prevalence in their countries of origin. Also Russian-born women have been shown

to have a higher cervical cancer incidence and a slightly lower cervical cancer screening participation than non-immigrant women. [104]

2.1.3.2 Cervical cancer subtypes

The two main histological subtypes of cervical cancers are squamous cell carcinoma and adenocarcinoma. Globally around 83% of all cervical cancers are squamous cell carcinomas, and 12% adenocarcinomas. [105] Around 2% of cervical cancers are histologically mixed adenosquamous carcinomas. [106] Cervical cancer screening has effectively decreased the prevalence squamous cell carcinomas in many countries, while the incidence of adenocarcinomas has simultaneously increased. [107]

A recent systematic analysis estimating the global HPV genotype-specific attributable fractions (AFs), meaning the proportions of cervical cancers causally attributable to each HPV type, identified 17 HPV types as causative. In addition to the 13 genotypes classified as carcinogenic or probably carcinogenic by IARC, see Table 1, the analysis also recognised four genotypes (HPV73, 26, 69, and 82) classified by IARC as possibly carcinogenic, as having limited carcinogenic potential. Across all histological subtypes, HPV16 had the highest AF at 61.7%, followed by HPV18 (15.3%), HPV45 (4.8%), HPV33 (3.8%), HPV58 (3.5%), HPV31 (2.8%), and HPV52 (2.8%). The AF of HPV16 was higher in squamous cell carcinomas (63.7%) than in adenocarcinomas or adenosquamous carcinomas (46.6%). Conversely, the AF of HPV18 was greater in adenocarcinomas and adenosquamous carcinomas (38.5%) than in squamous cell carcinomas (13.2%). [19]

Survival rates for cervical cancer are similar across squamous cell carcinoma, adenosquamous carcinoma, and adenocarcinoma, with unadjusted five-year survival ranging between 55% and 65%. [108]

2.1.3.3 HPV-negative cervical cancers

HPV-negative cervical cancers do exist and are estimated to account for approximately 7–11% of all cervical cancer cases. [109–111] These cancers have been associated with poorer survival outcomes compared to HPV-positive cervical cancers. [112,113] However, a proportion of HPV-negative cases are likely false negatives, attributable to factors such as histological misclassification, latent HPV infection, disruption of the targeted genomic region during HPV integration, or infection with an HPV genotype not detected by the test used. [114] In a recent Swedish quality assurance study, 43% of HPV PCR-negative cancers had HPV DNA, when analysed with deep sequencing. [115] As detection methods continue to evolve, it is likely that a greater number of these HPV-negative cases will be reclassified as false negatives. Most truly HPV-negative cervical cancers, based on currently available detection methods, are rare adenocarcinoma subtypes, including gastric, clear cell, serous, and mesonephric adenocarcinomas. [116]

2.1.4 Other HPV-related anogenital cancers

Other HPV-related anogenital cancers, including vulvar, vaginal, and anal cancer, are relatively rare worldwide. [117] Increase in the incidences of anal and vulvar cancer have been reported during the recent decades in multiple high-resource countries. [10,11,118–121] Increased HPV prevalence due to generational changes in sexual behaviour is likely associated with these incidence changes. [122] As especially vulvar and vaginal cancers are common among older individuals, the aging and growing world population will likely contribute to increasing anogenital cancer amounts in the near decades. [123]

All HPV-mediated anogenital cancers have precancerous lesions similar to those in cervical cancer, yet the AF of HPV differs among these cancers. [117] Other risk factors for these cancers are similar to cervical cancer, including smoking, and immunosuppression. [124–126] As for cervical malignancies, prior cervical neoplasia is also a risk factor for all of these cancers and their precancerous lesions. [57,127]

2.1.4.1 Vaginal cancer

Around 78% of vaginal cancers are attributable to HPV. As vaginal cancer is one of the rarest gynaecologic malignancies, this means only 12,000 new cases per year worldwide. [117] Of all HPV-positive vaginal cancers, around 64% are attributable to the genotypes HPV16 or HPV18, and 20% to the genotypes HPV31, HPV33, HPV45, HPV52, and HPV58. [128]

The overall survival of vaginal cancer is worse than the survival of cervical cancer, but HPV-associated vaginal cancers have a better prognosis than the ones with different aetiology. [129,130]

Vaginal precancers were previously categorised into vaginal intraepithelial neoplasia (VaIN) grade 1, 2, and 3 similarly as cervical precancers, but the two-tiered classification into vaginal LSILs and HSILs is now recommended. [88] Vaginal precancer incidence is on estimate 100-fold less than that of cervical precancers. [131] HPV prevalence in vaginal HSIL cases is around 96%. [128] Around 7% of VaIN3s progress to invasive vaginal cancer. [132] For VaIN1, the recommended treatment modality is active surveillance, for VaIN2 laser therapy or excision, and for VaIN3 excision. [132]

2.1.4.2 Vulvar cancer

Vulvar cancer is more common than vaginal cancer, with an estimated number of 34 000 incident cases per year globally. Nevertheless, the proportion of HPV-associated cancers is lower in vulvar cancer (25%), and thus the number of incident HPV-associated vulvar cancers per year globally is only 8 500, less than the number of HPV-associated vaginal cancers. [117] The contribution of HPV16 or HPV18 to the HPV-positive vulvar cancer is 72.6% and the contribution of HPV31, HPV33,

HPV45, HPV52, or HPV58 13%. [128] The HPV association in vulvar cancers is more common among younger vulvar cancer patients. [133] As vulvar cancer incidence is rising mainly among younger individuals [134], increased HPV prevalence is a plausible explanation behind the incidence increase. Similarly, as in vaginal cancer, the overall survival of HPV-associated vulvar cancer is better than non-HPV-associated vulvar cancer. [135]

Vulvar precancers are again categorised into vulvar intraepithelial neoplasia (VIN) grades 1,2, and 3, or into vulvar LSILs and HSILs. [88] The HPV prevalence in vulvar HSILs is around 87%, a lot higher than in vulvar cancers. [128] The progression rate of vulvar HSILs into vulvar cancer is around 13%. [136]

2.1.4.3 Anal cancer

Anal squamous cell carcinoma (ASCC), the most common anal cancer subtype, and ASCC incidence is increasing globally among both men and women. [12] Also an increase in ASCC mortality has been reported. [137] ASCC is also the anal cancer subtype associated with HPV, on estimate 91% of all ASCC cases being caused by HPV. [138] HPV16 is the type found in 83% of HPV-positive ASCCs and also 83% of anal precancers. The other HPV genotypes have a significantly smaller contribution to the anal malignancies than to the other anogenital malignancies. [128]

Anal cancer is particularly common among men having sex with men, but as mentioned above its incidence is rising among women as well. Other risk factors for anal cancer include human immunodeficiency virus (HIV), immunosuppression due to solid organ transplants, and a history of gynaecological malignancies. [122,139] The estimated number of incident anal cancers per year attributable to HPV among women is 18 000. [117]

Anal HSIL incidence among HIV-negative women has been estimated to be around 2%, but the research on the topic is limited. [140] Based on a recent study, anal HSILs of people living with HIV should be treated. [141] There is no clear consensus on the management of anal HSILs among HIV-negative women, but as a history of gynaecologic neoplasm increases anal cancer risk [142], treating the anal HSILs among individuals in this risk group could be justified.

As the progression rate from HSIL to anal cancer is high among individuals living with HIV, also screening for anal cancer among this risk group is justified. [141]

2.2 Cancer screening

Cancer screening is a form of secondary prevention aimed at detecting and treating cancers or precancerous lesions at an early stage to prevent their progression. The primary objective of cancer screening is to reduce the mortality and morbidity associated with the screened cancer. When a detectable precursor exists, screening can also reduce the cancer incidence by identifying and managing precancerous changes before they develop into cancers.

2.2.1.1 Principles

The most known and still widely used principles for disease screening by Wilson and Jungner were published in 1968, over 60 years ago. [143] A 2018-published systematic review and consensus process of screening principles aimed to update them to be more suitable for the modern times. [144] This review found the principles of Wilson and Jungner to be remarkably enduring, while also updating them to incorporate new aspects related to screening programme and system considerations. Additionally, some of the original principles were slightly adjusted. The 12 updated principles are presented in Table 3.

Table 3 Updated screening principles, based on a systematic review and modified Delphi consensus process), modified from Dobrow et al. 2018. [144]

Domain	Updated screening principles
Disease principles	1. Epidemiology of the disease should be adequately understood, and the disease should be an important health problem
	2. The natural history of the disease should be adequately understood, the disease should be well-defined, and there should be a detectable preclinical phase
	3. The target population for screening should be clearly defined, identifiable, and able to be reached
Test/intervention principles	4. Screening test performance should be appropriate, test should be accurate, reliable or reproducible, accepted by the target population, safe, affordable, and efficient
	5. Screening test results should be clearly interpretable and allow identification of the screening participants who should receive further testing or care
	6. There should be an agreed course of action for screening participants with positive screening test results that involves diagnostic testing, treatment or intervention, and follow-up care
Program/system principles	7. Adequate existing infrastructure or a clear plan to develop adequate infrastructure, that is appropriate to the setting
	8. All components of the screening programme should be coordinated and, where possible, integrated with the broader health care system to optimize care continuity and ensure no screening participant is neglected
	9. All components of the screening programme should be clinically, socially, and ethically acceptable. There should be effective methods for providing screening participants with informed choice
	10. High-quality scientific evidence should indicate that the overall benefit of the screening programme outweighs its potential harms
	11. An economic evaluation of the screening programme, using a health system or societal perspective, should be conducted
	12. The screening programme should have clear goals or objectives that are explicitly linked to programme planning, monitoring, evaluating and reporting activities

2.2.1.2 Evidence of effectiveness

According to the updated principle 10, before implementing a new cancer screening program, there must be clear evidence that its benefits outweigh its potential harms. The primary benefits of cancer screening include reduced mortality and morbidity and, in some cases, a decrease in cancer incidence, as previously mentioned. When these benefits are evaluated under ideal, controlled conditions, the study provides an estimate of the efficacy of the screening program. However, in public health and clinical practice, the primary concern is how well screening programs perform in a real-world setting. This is referred to as the effectiveness of a screening program.

Randomised-controlled trials (RCTs) are the gold-standard study design when it comes to assessing the effectiveness of a cancer screening program. RCTs have major advantages compared to non-randomised study designs, most importantly a correctly implemented randomisation eliminates selection bias. In this case the randomised groups are homogenous, which controls also for the possible unknown confounders. The random allocation also enables blinded study designs, which reduce bias in the results. Properly conducted observational studies, however, can also provide reliable estimates on the screening effectiveness. [145]

2.2.1.3 Harms of cancer screening programmes

The potential harms of a screening programme include overdiagnosis, overtreatment, psychological harms, and physical complications. In epidemiology, overdiagnosis is defined as the diagnosis of a condition that would have remained indolent in the patient's lifetime if left undetected. [146] In cancer screening, this means precancerous lesions or cancers that will not progress into more advanced forms of disease and cause no harm to the individual. [147] Some of these overdiagnosed cases could even regress on their own.

The main consequence of overdiagnosis is overtreatment. Overtreatment can cause unnecessary adverse effects caused by the treatment itself, burden healthcare systems, cause unnecessary costs, and derail resources from patients who actually need the treatment. Overdiagnosis itself can also cause psychological distress for the diagnosed individual. [148]

Otherwise, psychological distress among the cancer screening participants is low during the screening process and, thus, might not be a widespread barrier to screening participation. [149] Fear of the screening process, however, can be a barrier. On the other hand, anticipatory anxiety about a cancer diagnosis might even increase the screening participation. [150]

The physical complications associated with cancer screening programs vary depending on the type of cancer being screened. I will mention the most common complications caused by cervical cancer screening programs in further chapters.

2.3 Cervical cancer screening

In many countries, cervical cancer screening programs were the first implemented cancer screening programs. First community-based screening programs started already in the 1940s in the United States [151,152], after Georges Papanicolaou had invented the Papanicolaou test (Pap test). [153]

2.3.1 Cytology screening

Cytology screening is based on the Pap test, in which the sample-taker collects cervical cells using a small brush or spatula. In conventional cytology screening, the cervical cells are either placed directly onto a glass slide or first transferred to a liquid medium before being applied to the slide. The cells are then stained, after which a cytotechnician evaluates them under a microscope. Slides with abnormal findings are further reviewed by a cytologist or cytopathologist. Evaluating the cells is complex and requires a trained professional, which is one of the disadvantages of cytology screening. [154]

The most widely used classification system for cytological findings today is the Bethesda System (TBS) [155], which was also the base for the two-tiered classification of histologically confirmed anogenital precancers. [88] Other systems have also been used, for example, the Papanicolaou classification system [153], employed in Finland until the early 2010s, before TBS was adopted. Table 4 presents the classification of cytological findings in TBS and Papanicolaou classification system and their approximate correspondence.

Table 4 Classification of cytological findings and their approximate correspondence in the Papanicolaou system and the Bethesda System

*The Papanicolaou class II does not directly correspond to ASCUS/AGCNOS, as the class II also includes reactive non-malignant changes in the Papanicolaou classification.

Papanicolaou class	the Bethesda System
I	NILM (negative for intraepithelial lesion or malignancy)
II*	ASCUS (atypical glandular cells of undetermined significance)/AGCNOS (atypical glandular cells of undetermined significance)
III	LSIL (low-grade intraepithelial neoplasia)
IV	HSIL (high-grade intraepithelial neoplasia)
V	Cancer

2.3.1.1 Liquid-based cytology

A more modern version of cytological screening is liquid-based cytology (LBC), which was developed in the 1990s. In LBC, cervical cells collected with a broom are first rinsed into a liquid buffer solution, after which some of the cells are placed onto the glass slide for analysis. The cells can also be stored in the buffer solution, and for example an HPV test can later be performed from the same LBC sample. [156] The proportion of unsatisfactory samples is lower in LBC compared to conventional cytology, and its interpretation takes less time, but the use of LBC also costs more than conventional cytology. However, the effectiveness of LBC and conventional cytology are similar and thus the use of conventional cytology is still justified. [157]

2.3.1.2 Effectiveness of cytology screening

The efficacy of cytological screening was not evaluated in randomised controlled trials (RCTs) prior to its implementation. Consequently, evidence of its effectiveness is based solely on observational studies. These include cohort and case-control studies, as well as reports on cervical cancer incidence and mortality trends following the introduction of screening programmes. Cytology screening has proven effective in reducing both cervical cancer incidence and mortality in all countries with established population-based screening programmes. [158] One of the most convincing pieces of evidence for the effectiveness of cytology screening came from Finland, where a study reported an 80% reduction in cervical cancer incidence among women who participated in the screening programme. [159]

2.3.2 HPV screening

HPV test-based cervical cancer screening, hereafter referred to as HPV screening, is based on the detection of high-risk HPV genotype DNA or RNA from cervical cells. Current criteria for HPV tests used in screening require that they detect the 12 genotypes classified by IARC as carcinogenic to humans (see Table 1). [160]

The development of HPV tests for screening began shortly after the causation between HPV and cervical cancer was established, and the first HPV tests were developed already in the 1980s. [161] Large-scale RCTs to establish the effectiveness of HPV screening compared to cytology screening started in the early 2000s.

2.3.2.1 Effectiveness of HPV screening

The RCTs provided convincing evidence that HPV screening is more sensitive for detecting high-grade cervical lesions and identifies them earlier than cytology screening. [5–7,162] A pooled analysis of these RCTs demonstrated a 60–70% greater protection against invasive cervical cancer over a median follow-up of 6.5 years with HPV screening compared to cytology. This pooled study also noted a greater reduction in the incidence of adenocarcinoma compared to squamous-cell carcinoma with HPV screening. [9] HPV screening has also shown to have a higher negative predictive value (NPV) for HSILs than cytology, allowing for longer screening intervals with HPV testing. [163]

Based on this evidence, the European Guidelines for Quality Assurance in Cervical Cancer Screening recommended transitioning to primary HPV screening in 2015. [4] However, long-term data on the effectiveness of HPV screening in reducing cervical cancer incidence and, particularly, cervical cancer mortality, remain limited.

2.3.2.2 Implementation

HPV screening also offers several advantages over cytology screening. The results of HPV tests are faster and easier to analyse, and the analysis does not require highly trained healthcare personnel. Furthermore, HPV screening is less expensive than cytology screening, making it more feasible in low-resource countries. [164] Additionally, HPV screening allows for self-sampling, which can increase screening coverage, and help reach demographic with low participation in screening programmes. [165,166]

A 2022 review found that 48 out of 139 countries studied had implemented primary HPV screening. The proportion of countries using HPV screening was highest among high-income countries, with 25 out of 56 (45%) having adopted HPV screening. [3] One of these countries was Finland, where the gradual implementation of HPV screening to the population-based screening programme began in 2012. By

2022, almost 80% of all tests in the Finnish cervical cancer screening programme were HPV tests. [167]

2.3.2.3 Harms of HPV screening

The increased detection of cervical lesions in the HPV screening arms of RCTs raised a concern of overdiagnosis instead of early detection. [5,168] However, a longer follow-up data from RCTs has shown indications that the amount of detected lesions decreases after the initial HPV screening round, which indicates towards early detection. [163] Observational studies have also reported lower HSIL+ rates among HPV screened in the subsequent screening rounds. [14,169]

Nevertheless, increased detection of especially low- and moderate-grade cervical lesions during the initial screening rounds can lead to overtreatment. This is especially concerning among the youngest age groups in HPV screening, as they are always having their first HPV-screening round when they enter the HPV screening program. The adverse effects of precancer treatments also affect individuals in reproductive age more as the most common way to treat cervical precancers are excisional procedures, and the adverse effects of these treatments are mainly obstetric. The adverse obstetric effects include preterm birth, low-birth weight, and even perinatal mortality. [170,171]

For cervical LSIL lesions the recommended management option has been active follow-up since 2006, before which all LSIL cases were also treated. [172,173] Due to the possible obstetric adverse effects, higher regression rate and lower progression rate of HSILs corresponding to CIN2 among younger individuals, many countries have started to recommend their conservative management among individuals younger than 25. [174] The Finnish guidelines currently recommend active surveillance of CIN2 lesions among individuals younger than 31. [175] Previously it has been shown that among individuals aged 25 to 30 years, the CIN2 regression rate is much higher, than the average across all ages, around 72%. [176] However, a recent Finnish study did not find age (unlike lesion size, the presence of carcinogenic HPV genotypes, and cytology) to be associated with a higher likelihood of lesion regression.[177] An appropriate management strategy for cervical precancers is essential to minimise the harms of HPV screening, and further research is needed to determine the optimal approach.

Many high-income countries, having implemented primary HPV screening, have also reported increased colposcopy referral rates. This creates burden to the healthcare system, as performing a colposcopy requires a trained gynaecologist, and can in resource-limited settings create longer waiting times for the individuals with an actual urgent need for a colposcopy. Unnecessary colposcopies can also lead to overdiagnosis of regressive cervical lesions, as explained above. A Danish study reported a 6.4% colposcopy referral rate in HPV screening, compared to the 2.3% referral rate in cytology screening. The rate was especially high among the age group

30-39, 8.4% in HPV screening, compared to the 3.3% in cytology screening. [14] Results from the HPV screening programme in Umbria region Italy also reported an increase in colposcopy referral rate, and even though the rate decreased in the second screening round, it was still 3.7% compared to the 1% in cytology screening. [178] A Finnish study on the colposcopy referral rate after two screening rounds with primary HPV screening in a regional screening programme, also noticed a lower colposcopy rate during the second screening round than in the first round (4.0% vs. 2.9%). [16] In this study, the studied cohort remained the same across both rounds, which does not accurately reflect real-world programmes, where new age cohorts with highest HPV prevalence enter HPV screening in each round.

2.3.2.4 HPV screening algorithms

The most effective way to tackle the increased colposcopy referral rates in HPV screening is to create screening algorithms correctly identifying progressive HPV infections and existing precancers, from non-progressive infections. How well the screening algorithm works depends on multiple factors including the screening interval, referral criteria, and HPV prevalence in the screened population. One of the most important things when it comes to reducing the increase in colposcopy referrals is the triage method for HPV-positive individuals.

2.3.2.5 Cytology triage

Currently, the most common triage is cytology, with 38% of countries with primary HPV screening programs recommending its use. [3] European Guidelines for the quality assurance of cervical cancer screening also recommend cytology triage for those who test HPV-positive. [4] All of the above-mentioned colposcopy referral rates were from primary HPV screening programmes using cytology triage for HPV-positive individuals. [14,16,178] This gives indication that cytology triage might not be sufficient to increase the specificity of HPV screening to an optimal level. The colposcopy referral rates, however, also depend on the decisions done based on the cytology results.

In Finland, currently recommended management is to send HPV-positive individuals with a cytology triage result of LSIL, or worse straight to colposcopy and individuals with a normal or ASCUS cytology to HPV and cytology retests after 18 to 24 months. [179] Like in the Finnish algorithm, in many HPV screening algorithms repeatedly HPV-positive individuals with a normal cytology are referred to colposcopy, which causes a significant portion of the increased colposcopy referral rates. [180] What would be the optimal management of these HPV-positive individuals with normal cytology has not, however, been assessed.

2.3.2.6 HPV genotyping triage

Another triage method for HPV-positive individuals is HPV genotyping. The incorporation of HPV genotyping to screening programmes started with partial genotyping, where HPV infections are categorized to HPV16/HPV18 and other. As more knowledge on the differences in carcinogenicity between the types previously classified as "other" grew, and the genotyping tests became less expensive and more accessible, extended genotyping started to be more feasible in screening programmes. Extended genotyping refers to assays reporting at least six individual high-risk HPV genotypes. Assays that report all high-risk HPV genotypes individually are referred to as full genotyping. [181,182] Table 5 presents the three different genotyping levels and gives examples on assays which they can be performed with.

Table 5 Different HPV genotyping levels and their definitions. The assay examples are based on the list provided in Arbyn et al. 2021. [183]

Genotyping	Definition	Assay examples
Partial	Reports HPV16 and HPV18, other carcinogenic HPVs pooled	Abbott RealTime Cobas 6800
Extended	Individually reports at least 6 carcinogenic HPVs genotypes and the remaining in one or more groups	BD Onclarity
Full	Individually reports all carcinogenic HPVs genotypes	Luminex

Different risk classifications for the genotypes have been proposed. A study from the United States suggested grouping HPV genotyping results into five risk groups (HPV16, HPV18/45, HPV31/33/58/52, HPV51/35/39/68/56/66/68, HPV-negative) based on their precancer and cancer risk. [184] HPV18 has been thought to be the second most high-risk genotype for cervical precancers and cancers, but studies have shown that HPV31, and HPV33 have a similar, or even higher HSIL+ risk. [18,185] Thus, it is not clear how the results from extended genotyping should be managed in screening programmes.

A promising approach is also to combine HPV genotyping with cytology triage. [186] A 2020 systematic review concluded that HPV extended genotyping has clinical utility in the risk stratification of cytology results. [187] What are the exact long-term risks each genotype and cytology triage result possess for cervical precancers, and cancers have, however, not been established. We also lack long-term studies of the genotype-specific risks stratified by age.

2.3.2.7 Other triage methods

In normal cells the proteins p16 and Ki-67 are not expressed at the same time, but during carcinogenesis the HPV E7 protein upregulates p16 to be continuously expressed. Thus dual staining cytological slides with p16/Ki-67 shows that malignant transformation of cervical cells has been initiated. [188,189] Dual staining has been shown to provide better risk-stratification than cytology among HPV-positive women, both when combined with partial HPV genotyping and alone. [190]

DNA methylation of both host and viral genes is well established in cervical carcinogenesis, and DNA methylation markers are a feasible triage method in HPV screening, as they are implementable also to self-collected samples. [191] Several recent studies have assessed the potential of different DNA methylation panels in cervical cancer screening, and results are promising. A recent Swedish study showed that combining partial HPV genotyping with a host gene methylation panel outperformed cytology for triaging HPV positive women. [192] Other study on a randomised HPV screening cohort showed increased sensitivity for identifying prevalent CIN3 cases with a combined host and virus gene DNA methylation panel compared to partial genotyping, although the panel showed lower specificity than partial genotyping. [193] A study on a cohort of HPV vaccinated young women showed low DNA methylation levels in vaccinated women with HSIL lesions attributed to lower risk types, which gives indications on the potential of DNA methylation in risk-stratification among vaccinated cohorts. [194]

2.4 HPV vaccination

HPV vaccination reduces the incidence of cervical cancer substantially among vaccinated individuals. The effect is strongest among individuals vaccinated at a young age, before acquiring HPV infections. [195] Herd effects against high-grade cervical lesions have also been demonstrated in settings with multi-cohort vaccination and high routine vaccination coverage. [196] In addition to cervical cancer, HPV vaccines are effective against other HPV-related malignancies; although the impact on invasive cancers at non-cervical sites has yet to be fully established, reductions in vaginal, vulval, and anal high-grade squamous intraepithelial lesions have been reported. [197,198] Thus, HPV vaccination is the most effective strategy to reduce HPV-related malignancies in the future.

There are however several open questions related to HPV vaccination, one of which concerns the potential for type replacement and its consequences. A recent Finnish study observed increased ecological diversity of *Alphapapillomaviridae* genotypes, in a cohort of vaccinated individuals. As vaccine-targeted genotypes became less prevalent, other genotypes appeared to increase in prevalence. [199] What this means for the future risk of HPV-related malignancies remains to be determined.

2.4.1 Screening in the vaccination era

In 2020, the World Health Organization (WHO) released the *Global Strategy to Accelerate the Elimination of Cervical Cancer as a Public Health Problem*. The strategy outlines three global targets to be achieved by 2030: 90% of girls fully vaccinated against HPV by the age of 15; 70% of women screened with a high-performance test by the ages of 35 and 45; and 90% of women identified with cervical disease receiving appropriate treatment. [200] These targets, however, seem somewhat ambitious. A 2022 review estimated that two in three women aged 30–49 years have never been screened for cervical cancer. [3] Recent studies indicate that single-dose HPV vaccination offers comparable effectiveness to the three-dose strategy, which has a potential to improve the global vaccination coverage. [201,202] Nonetheless, even single-dose coverage remains well below the target, reaching only 27% in 2023. [2]

In Finland, the routine HPV vaccination programme started in 2013, and the first age cohort vaccinated was born in 1998. It expanded to a gender-neutral programme in 2020. [203] Current vaccination coverage among girls is approximately 80%, which remains below the WHO target. [204]

Continued efforts are therefore essential to increase vaccination uptake, extend cervical cancer screening to regions without established programmes, and improve participation in areas where screening is already in place.

Nevertheless, vaccination will change the cervical cancer screening programs drastically in the upcoming decades. Depending on vaccination coverage and the vaccine used, the vaccinated population does not necessarily need a population-based screening programme for cervical cancer, or a de-intensified programme might suit the population better. [205] In Finland the first vaccinated cohort will turn 30 and thus enter the cervical cancer screening programme nationwide in 2028. In the vaccinated cohorts, HPV screening has been estimated to be more cost-effective than cytology screening, which is another possible advantage of HPV screening. [206,207]

2.5 Screening for HPV-related anogenital cancers

Screening for vaginal, vulvar, or anal cancer is not recommended in the general population due to their rarity. Screening for high-risk individuals has, however, been proposed, and in some cases already implemented.

A recent consensus guideline report by International Anal Neoplasia Society recommends anal cancer screening for various high-risk groups, including people living with HIV, solid organ transplant recipients, people with autoimmune conditions, and people with history of HPV-related gynaecological malignancies. The recommended primary screening methods are anal cytology and anal HPV

testing. [208] Whether primary cervical HPV screening could have a beneficial effect on anal cancer incidence by increasing the detection and treatment of gynaecological HPV-related lesions or by increasing the detection of HPV-related anal lesions is unknown.

In some countries, like in Finland, continuing cervical cancer screening for hysterectomised individuals has been justified as a possibility to prevent vaginal and vulvar cancers, by detecting their precursors during the screening process. [209] Screening individuals with a hysterectomy due to benign conditions, and not due to cervical malignancies is, however, not effective based on the current evidence. [210] Additionally, the cytological screening has not led to decreased incidences of these cancers. [130] Vaginal cancer screening for individuals with a history of cervical precancer or cancer, regardless of their hysterectomy status, with an HPV test has been proposed. [210] Nevertheless, how cervical HPV screening affects the incidence of vaginal and vulvar cancers has not been assessed among general population, or among high-risk individuals.

3 Aims of the study

The first general aim of this thesis was to assess the benefits of primary HPV test based cervical cancer screening in comparison to primary cytology screening. The second general aim was to find ways to tackle the biggest harm of HPV screening, its lower specificity compared to cytology screening. The particular aims of each sub study are presented below.

1. To assess the long-term effectiveness of primary HPV screening on cervical cancer incidence and mortality (I)
2. To examine the possible additional benefit of cervical HPV screening on cancer incidence and mortality of other anogenital HPV-related cancers (II)
3. To find the optimal algorithms for HPV screening with cytology triage by assessing their sensitivities and specificities for HSIL+ (III)
4. To assess how different HPV genotypes should be managed in an HPV screening programme by establishing the long-term risks they carry for HSIL+ (IV)

4 Material and methods

4.1 The Finnish HPV screening trial

This thesis is based on the long-term follow-up data from the Finnish randomised HPV screening trial, organised as a part of the population-based cervical cancer screening programme. [168,211–213] The trial is registered as an International Standard Randomized Controlled Trial with the number ISRCTN23885553. [214] In nine municipalities of Southern Finland (Porvoo, Järvenpää, Tuusula, Vantaa, Hyvinkää, Lohja, Kirkkonummi, Espoo, and Helsinki) 236 727 individuals from the cervical cancer screening target population were randomised with 1:1 ratio to either HPV or cytology screening. The National Authority of Medicolegal Affairs, the participating municipalities' health boards, and the ethical committee of the regional hospital district approved the trial before its initiation in 2003. The trial lasted for two five-year screening rounds, until 2013.

4.1.1 Trial protocol

During the trial, the Finnish cervical cancer screening programme was a nation-wide programme in which municipalities were responsible for the practical implementation and associated costs. In most municipalities, the target age range for screening was 30 to 60 years; however, Helsinki also included individuals aged 25 and 65. The trial was integrated into the existing screening programme, and consequently, the age groups targeted during the trial corresponded to those already adopted by the municipalities.

Individuals from the screening target population were identified from the national Population Register and the Finnish Cancer Registry personnel generated the random allocation sequences using computer-assigned random numbers and allocated the study participants to the study arms. Municipalities sent the screening invitations as in the routine screening program. Each randomised individual received a similar personal letter in mail together with a special information brochure of the trial. Individual randomisation status was not revealed in the letter, but it was registered to the Screening Register. In both of the screening arms the samples were taken by a trained nurse or midwife, which is the common practise in the cervical cancer screening programme. The screening results were mailed from

the screening laboratories to the individual via physical mail. If colposcopy was indicated, the first contact was generally by phone. [212]

4.1.1.1 HPV screening arm

HPV test used in the HPV arm was Hybrid Capture 2 (HC2) and the triage method for HPV-positive individuals was conventional cytology. Thus, in the HPV screening arm the nurse or midwife took two separate samples from each attendee, an HPV sample with HC2 and a traditional cytological smear for the triage test. The smears were only analysed in the HPV screening arm if the HPV test was positive. HPV test results were not blinded from the laboratory and hospital personnel at any stages of the screening.

If the HPV test was positive and the cytology triage Pap class III, IV, or V individual was immediately referred for colposcopy and biopsy. This included cases of ASC-H, LSIL, HSIL and glandular atypia. If the HPV test was positive, and cytology triage Pap class I or II individual was referred to intensified screening. This meant a rescreen after 12 months from the initial visit, following similar protocol as in the initial screen. If HPV test was still positive and cytology triage showed no dysplastic changes, another rescreen after 24 months from the initial test was recommended. If the HPV infection persisted in the final rescreen, individual was referred to colposcopy, regardless of the cytology triage result. [212]

4.1.1.2 Cytology screening arm

The trial protocol in the cytology screening arm followed the standard cytology screening practices used in Finland. A cytology test result of Pap class III, IV, or V (including ASC-H, LSIL, HSIL and glandular atypia) led to a referral for colposcopy and biopsy. Individuals with a Pap class I result (normal cytology) were returned to routine screening, while those with a Pap class II result were invited for intensified screening after one year. [212]

4.1.1.3 The second screening round

The largest municipality of Finland, Helsinki, decided not to continue with the trial protocol for the second screening round, and returned to the routine cytology screening programme after completing one round in the trial. All other municipalities continued with the previously described trial protocol. No new individuals were randomised during the second screening round, and thus all individuals were five years older in the second screening round, and the oldest age group had exited the screening.

4.2 Study data

The long-term follow-up data from the HPV screening trial is based on individual-level data from multiple different data sources, of which most were existing population-based registries. Additionally, we collected the genotyping data of a total of 5 253 HC2 positive HPV samples from the HPV screening arm of the trial.

4.2.1 Register-based data

The history of population-based registries is long in Finland, dating back to 1952 when the first population-based register, the Finnish Cancer Register, was established. [215] All of the below mentioned register-based data were linked using personal identifiers assigned to all permanent residents.

4.2.1.1 The Finnish Cancer Register (I-IV)

The Finnish Cancer Register (FCR) is a national register of all individuals with a cancer diagnosis in Finland. The register includes cancer cases from the year 1953 onwards, and covers, on estimate, 96% of all solid tumour cases. [216] From the year 2008 onwards, the cancer type has been classified based on the International Classification of Diseases for Oncology third edition, ICD-O-3 coding system. [217] All previously recorded cases were also converted to this ICD-O-3 format. FCR also has data on the causes of death for individuals diagnosed with cancer, which is supplemented from the Statistics Finland. Some precancer cases, for example a portion of cervical HSILs, are also reported and recorded to FCR.

For the individuals randomised to the trial, we had data from the Finnish Cancer Register on all recorded cases from 1953 onwards of cervical cancer (ICD-O-3 topography code C53), cervical precancer and adenocarcinoma in situ, vaginal cancer (C52), vulvar cancer (C51), and anal cancer (C21), including information on deaths caused by these cancers. For studies I–III, cancer data were available up to the end of 2020, and for Study IV, cancer data were available up to the end of 2021.

4.2.1.2 The Screening Register (I-IV)

The Screening Register is part of the FCR and includes data on all of the three national cancer screening programmes (cervical, breast, and colorectal) in Finland. The cervical cancer screening data includes invitations, screening tests, their results, and diagnostics findings. Data is available starting from the year 1991. The screening invitation coverage among the target screening population has been almost 100% since 2008. [218] We had also the Screening Register data for the individuals randomised to the trial up to the end of 2020 for the studies I-III, and up to the end of 2021 for the Study IV.

4.2.1.3 Care Register for Health Care (I-IV)

The Care Register for Health Care is a comprehensive database that collects information on health care services. [219] Our dataset included only services provided within public specialised health care. Data from the Care Register for Health Care were available from 1990 onwards for all randomised individuals. For Studies I and II, we obtained data on partial and complete hysterectomies performed in specialised health care. For Studies III and IV, data were available on cervical precancers identified through opportunistic testing or diagnostic visits within specialised health care. Additionally, for Study II, data on vaginal and vulvar precancers diagnosed in specialised health care were also included. For Studies I–III, data were available up to 2019, whereas for Study IV, data extended to 2021.

4.2.1.4 The Statistics Finland (I-IV)

The Statistics Finland is an official government agency responsible for producing and publishing statistics. [220] They are the main agency in Finland responsible for producing sociodemographic data. For the studies in this thesis, we received emigration data, sociodemographic data, and data of the causes of death for the individuals randomised to the trial. Similarly as the cancer data, for studies I–III, data from Statistics Finland were available up to the end of 2020, and for Study IV, data were available up to the end of 2021.

4.2.1.5 Tests outside screening programme (I, III)

Testing outside the population-based screening programme is common in Finland, on estimate 60% of all cervical screening tests are taken outside the screening programme. [221] Unlike the tests in the population-based cervical cancer screening programme, these tests are not registered to the Screening Register. However, as a part of a previous doctoral thesis, a dataset of these tests outside screening programme was collected, and this dataset was also available for this thesis work. [222] The dataset consists of cervical tests gathered from pathology laboratories and hospitals since 1980s, tests from the Student Health Services for the years 2000–2010, and 2012–2015, and tests covered by the Health Reimbursement Register. The data were the most comprehensive from 1996 until 2014, when health reimbursement records were available, but some tests were received until 2017.

4.2.2 HPV screening samples (IV)

At the trial screening visit, nurse or midwife collected cervical cells with the HC2 kit brush which was then placed in the HC2 transport medium tube. The cells were stored in the medium at -20°C. Of these samples, 5 253 HC2-positive samples were

genotyped and the results were used in the Study IV of this thesis. We linked the genotyping data using personal identification numbers to all of the previously mentioned individual-level register data used in the Study IV.

4.2.2.1 First genotyping batch

The HC2 HPV-positive samples from the first three years of the trial (2003–2005) were genotyped at the beginning of the 2010s with the Luminex assay, henceforth referred to as the first genotyping batch. The samples were originally genotyped for a Study by Leinonen et al., and more details of the genotyping procedure of the first batch samples can be found in that publication. [68] The Luminex assay has probes for HPV genotypes 6, 11, 16, 18, 26, 30, 31, 33, 35, 39, 40, 42, 43, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, 67, 68 (a and b), 69, 70, 73, 74, 81, 82, 83, 86, 87, 89, 90, 91, and 114. The first batch included valid genotyping results for 2 531 out of 2 611 samples (See Study IV, Figure 1).

4.2.2.2 Second genotyping batch

The second genotyping batch consisted of remaining HC2 HPV-positive samples from the first screening round of the trial. In 2023, we identified and collected 3001 HPV-positive entry test samples from 2006–2008. We delivered the samples to the International Human Papillomavirus Reference Center at Karolinska Institutet for genotyping analyses. The samples were genotyped with the BD Onclarity HPV genotyping assay. BD Onclarity detects six individual HPV genotypes (16, 18, 31, 45, 51, 52) and three HPV genotype groups (33/58, 35/39/68, 56/59/66). The laboratory also provided information on samples that contained low HPV18 DNA amounts, below the cut-off value of the assay, as detecting HPV18 with low viral loads enhances the sensitivity and specificity of HPV-based cervical screening. [223] These samples were considered as HPV18 positives, similarly to those with a higher than cut-off HPV18 viral load. Out of the 3001 samples in this batch, 2722 samples were valid in the genotyping analyses.

4.2.2.3 Harmonisation of the genotyping data

All genotyping data was harmonised to the BD Onclarity format. This means that all the possibly carcinogenic and non-carcinogenic genotypes detected with the Luminex assay were classified as HPV genotype negative, as well as all the samples that tested negative for all of the genotypes by BD Onclarity or by Luminex. Out the 2531 valid samples in the first genotyping batch, 1499 were positive for the BD Onclarity genotypes, and 1023 negative. Respectively, out of the 2722 valid samples in the second batch, 2196 were positive and 526 negative.

In the case of multiple genotypes present in the same sample, we classified each sample according to the genotype or genotype group with the highest HSIL+ risk based on our data, see Study IV Supplement 1 for details.

4.3 Study designs and statistical analyses

All data harmonisation, statistical analyses, and visualisations presented in the Results section of this thesis were performed with R: A language and environment for statistical computing. [224]

4.3.1 Assessing long-term effectiveness (I, II)

In Studies I and II, our goal was to assess the effectiveness of HPV screening compared to cytology screening on cervical and other anogenital cancer incidence and mortality.

4.3.1.1 Follow-up

In both studies, the individual-level follow-up started on the 1st of January of the year of the first screening invitation in the trial (further referred to as the entry invitation) since the exact invitation dates were not available. For Study I we excluded individuals who had died, emigrated, had a hysterectomy, or a diagnosis of cervical cancer prior to the entry invitation, see Study I, Figure 1. For Study II we excluded individuals who had died, emigrated, or had a diagnosis of vaginal or vulvar cancer prior to the entry invitation, see Study II, Figure 1.

In the cervical cancer incidence analysis of Study I, individuals were censored at the time of cervical cancer diagnosis, emigration, death, hysterectomy, or at the end of follow-up on 31 December 2020, whichever occurred first. In the mortality analysis, individuals were censored at death, emigration, or at the end of follow-up on 31 December 2020, whichever occurred first.

Study II included site-specific incidence and mortality analyses for vaginal and vulvar cancers, as well as pooled analyses of these two cancer sites. As a post hoc analysis, the study also included incidence and mortality analyses for anal cancer. In the site-specific analyses, individuals were censored at the diagnosis of cancer at the site in question, emigration, death, or on 31 December 2020, whichever occurred first. The pooled incidence analyses for vaginal and vulvar cancers followed the same approach as the site-specific analyses, except that follow-up ended at the diagnosis of the first vaginal or vulvar cancer, emigration, death, or on 31st of December 2020, whichever occurred first. In all mortality analyses, individuals were censored at emigration, death, or on 31st of December 2020.

4.3.1.2 Poisson regression

We used Poisson regression models to assess incidence and mortality rate ratios for the various outcomes, comparing the HPV screening arm to the cytology screening arm. A separate model was used for each outcome. The follow-up time contributed by each individual was converted into person-years, and the log of person-years at risk for the outcome in question was used as an offset in the models.

Age and calendar period were included as covariates in the Poisson regression models. Age was categorized based on the age at the time of the entry invitation. In Study I, the age groups were 25–30, 35–50, and 55–65 years. Ages within five-year intervals were rounded to the nearest number divisible by five. In Study II, the age groups were defined as younger than 35, 35 to 54, and 55 years or older. In both studies, the calendar period was divided into four groups: 2003–2007, 2008–2012, 2013–2017, and 2018–2020.

4.3.1.3 Cumulative incidence and mortality

Additional to the Poisson regression, we used Aalen-Johansen estimator to assess the cumulative incidence and mortality of cervical cancer in the study arms in Study I. [225] The Aalen-Johansen estimator is a non-parametric method used to estimate cumulative incidence functions while accounting for competing risks. In the cervical cancer incidence analysis the outcome was diagnosis of cervical cancer, censoring events were emigration, and hysterectomy, and death was a competing event. In the cervical cancer mortality analysis, death by cervical cancer was the outcome, and death from other causes was a competing event. All p-values for differences in cumulative incidence and mortality between the study arms were calculated with the Gray's method. [226]

4.3.1.4 Intention-to-treat principle

The main analyses in both Study I and Study II were conducted according to the intention-to-treat (ITT) principle. ITT refers to the practice of analysing data from randomised controlled trials based on the original randomisation groups, regardless of whether participants received the intended intervention. Adhering to this principle allows for the assessment of the real-world effectiveness of the intervention, as not all individuals necessarily adhere to the assigned intervention in practice. [227] It also helps to reduce bias, particularly selection bias, which can arise when analyses are restricted to those who comply with the intervention. In the studies included in this thesis, following the ITT principle meant including all randomised individuals in the incidence and mortality analyses in the screening arms they were assigned to, also those who mistakenly received the incorrect screening test or did not attend the trial screening test(s).

4.3.1.5 Sensitivity analyses

In addition to the ITT analyses, we performed sensitivity analyses in Studies I and II to further assess the validity of the results and the effects of possible confounding factors. In both studies we assessed the incidence and mortality rate ratios in the study arms restricted to the areas where the trial lasted for two screening rounds as a sensitivity analysis.

In the Study I we additionally assessed the incidence (IRR) and mortality rate ratios (MRR) separately for those who had a maximum of one test outside the screening programme during the trial period, as these tests are common in Finland and thus could have had an impact on the result. We also performed per protocol analyses as cumulative incidences by entry trial test attendance status and by entry test result.

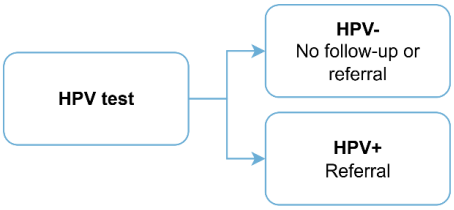
4.3.2 HPV screening algorithms with cytology triage (III)

In the Study III we created we created seven post-hoc screening algorithms based on the trial data, five different HPV screening algorithms with cytology triage, one HPV screening algorithm without triage, and cytology screening algorithm.

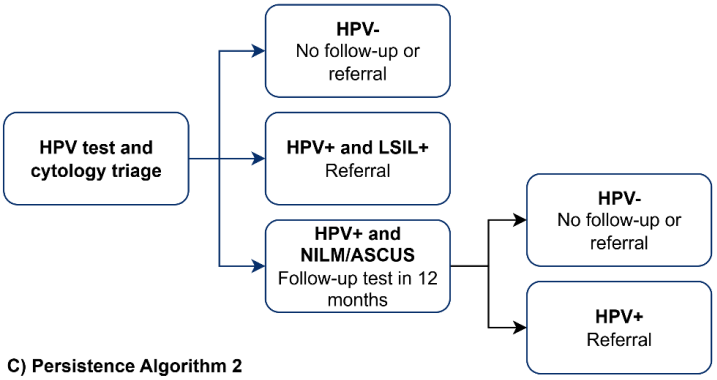
4.3.2.1 The post-hoc algorithms

All of the post-hoc algorithms are described in Figure 3 below. The algorithms with a cytology triage are comprised of different combinations of primary HPV test with a cytology triage and follow-up tests. The chosen algorithms were either used in HPV screening programmes, presented in previous studies, or otherwise easy to implement for screening with primary HPV screening. [209,228,229] The five algorithms for HPV screening with a cytology triage were classified into two HPV Persistence Algorithms and three Decisive Cytology Algorithms. The HPV Stand-alone Algorithm was an HPV screening algorithm without triage and Cytology Algorithm the routinely used cytology screening algorithm in Finland. The HPV Persistence Algorithm 2 and the Cytology Algorithm were those used in the trial.

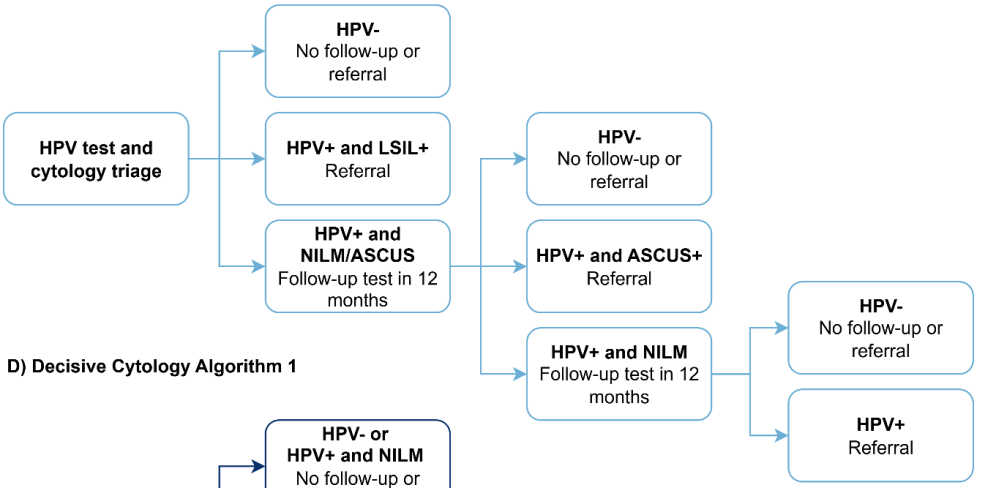
A) HPV Stand-alone algorithm



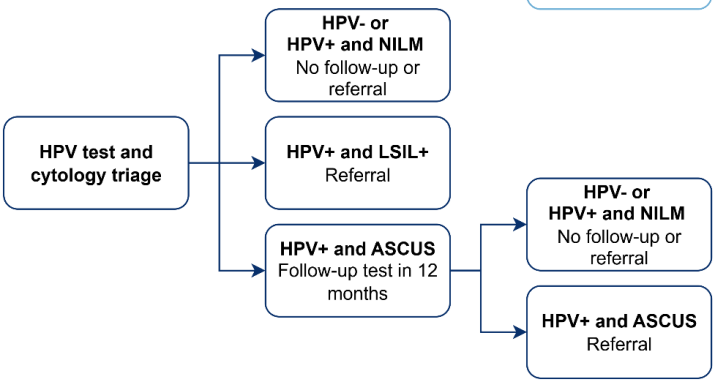
B) Persistence Algorithm 1



C) Persistence Algorithm 2



D) Decisive Cytology Algorithm 1



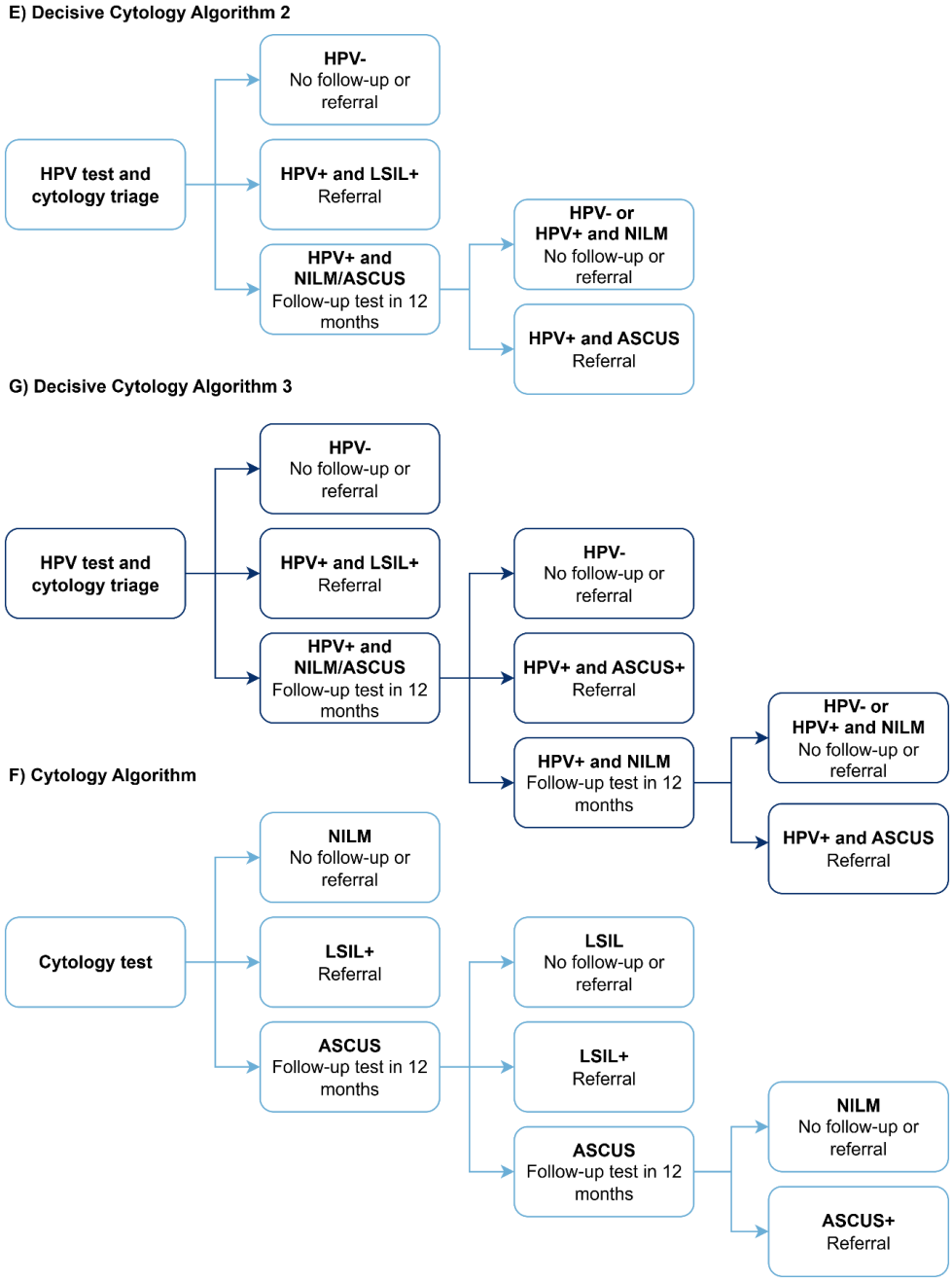


Figure 3 The post-hoc screening algorithms in Study III. Algorithm A) is a HPV screening algorithm with no triage, algorithms B)-E) are HPV screening algorithms with a cytology triage for HPV-positives, and algorithm F) is a primary cytology screening algorithm.

4.3.2.2 Algorithm characteristics

To characterise the post hoc algorithms, we assessed the episode (the screening round in this context) sensitivity for HSIL+, which episode specificity for HSIL+, positive predictive value (PPV) for HSIL+, and the colposcopy referral rate. These were assessed for all of the post hoc algorithms separately in the first and second screening rounds. Both screening rounds began at the entry test for the respective round and continued for 4.5 years. A screening finding that led to a referral at any point within the post hoc algorithm was considered a positive episode result. If the individual's screening round concluded without a referral, the episode result was considered negative.

Episode sensitivity was defined as the probability of a positive episode result given that the individual was diagnosed with HSIL+ during the episode. Episode specificity referred to the probability of a negative episode result given that the individual was not diagnosed with HSIL+ during the episode. The PPV represented the probability of a HSIL+ diagnosis given a positive episode result.

Colposcopy referral rates were also calculated according to the referral criteria specific to each algorithm. The diagnostic performance of the algorithms was subsequently illustrated using receiver operating characteristic (ROC) curves, generated separately for each screening round. Further details regarding the calculations and methodological considerations are provided in Study III.

4.3.3Long-term genotype-specific risks (IV)

In the Study IV our main outcome was HPV genotype-specific risks for HSIL+. We also assessed the genotype-specific risks separately for different age groups and cytology triage results. These risks were calculated as cumulative incidences.

4.3.3.1 Cumulative incidence

The cumulative incidences were calculated with the Aalen-Johansen estimator [225] using HSIL+ as an outcome. As in Study III the HSIL+ outcome included all HSIL+ cases diagnosed within and outside the screening programme.

In the analyses, for each HC2 positive genotyping positive individual the follow-up began on the date of the entry HPV test, where the genotyped HPV sample was taken, and continued until the earliest occurrence of a HSIL+ diagnosis, death, exit from the cervical cancer screening program, or the end of follow-up on December 31st, 2021. Of these death was the only event treated as a competing event. All of the p-values for cumulative incidence differences were calculated with

the Gray's method. [226] More details of the analyses are presented in the Materials & Methods section of Study IV.

The cytology triage results for the stratified analyses followed the Papanicolaou classification used in the trial: (1) negative (Papanicolaou class I), (2) ASCUS or AGCNOS (Papanicolaou class II), or (3) low-grade squamous intraepithelial lesion or worse (LSIL+) (Papanicolaou class III-V). In the age-specific analyses individuals were categorised into three groups based on their age at the entry HPV test: younger than 35, 35–49, and 50 or older.

5 Results

5.1 Long-term effectiveness of HPV screening

5.1.1 Long-term effectiveness on cervical cancer (I)

The study population of Study I consisted of 118 303 individuals randomised to two rounds of HPV screening and 118 129 individuals randomised to two rounds of cytological screening. The baseline characteristics between the study arms were similar, see Table 1 in Study I. As Helsinki did not continue with the trial protocol after the first screening round, only 50 997 (43.2%) individuals in the HPV arm and 50 950 (43.1%) individuals in the cytology arm were invited to the initially planned two screening rounds in the trial.

In the first screening round of the trial, 65.3% of individuals invited to the HPV screening arm attended, compared to 64.8% in the cytology arm. In the second screening round, attendance was 60.4% among those invited to the HPV arm and 60.2% among those invited to the cytology arm. In the HPV screening arm, the proportion of attendees who received an HPV test according to the protocol, rather than a primary cytology test, was 93.8% in the first round and slightly lower, at 80.8%, in the second round. Correspondingly, in the cytology screening arm, the coverage of cytology tests was 99.8% in the first round and 99.1% in the second round.

During the total of 3.5 million person-years of follow-up (1.74 million in each study arm) we detected 129 cervical cancers and 32 cervical cancer deaths in the cytology arm and 139 cervical cancers and 32 cervical cancer deaths in the HPV arm. The cumulative incidence and mortality of cervical cancer in the trial study arms are presented in Figure 4. There was no statistically significant difference between the study arms in cervical cancer incidence (IRR 1.08; 95% CI 0.85–1.37 in the HPV arm compared to cytology arm) or cervical cancer mortality (MRR 1.00; 95% CI 0.61–1.64 respectively) in the ITT analysis.

The sensitivity analyses confirmed this somewhat unexpected result. The analysis restricted to areas were the trial lasted for two screening rounds, showed an IRR of 0.91 (95% CI 0.63–1.29), and MRR of 0.83 (95% CI 0.35–1.93) in HPV

screening compared to cytology screening. The sensitivity analysis for those who had a maximum of one screening test outside the programme during the trial, IRR in the HPV arm compared to the cytology arm was 1.03 (95% CI 0.77–1.38), and the corresponding MRR was 0.97 (95% CI 0.57–1.67).

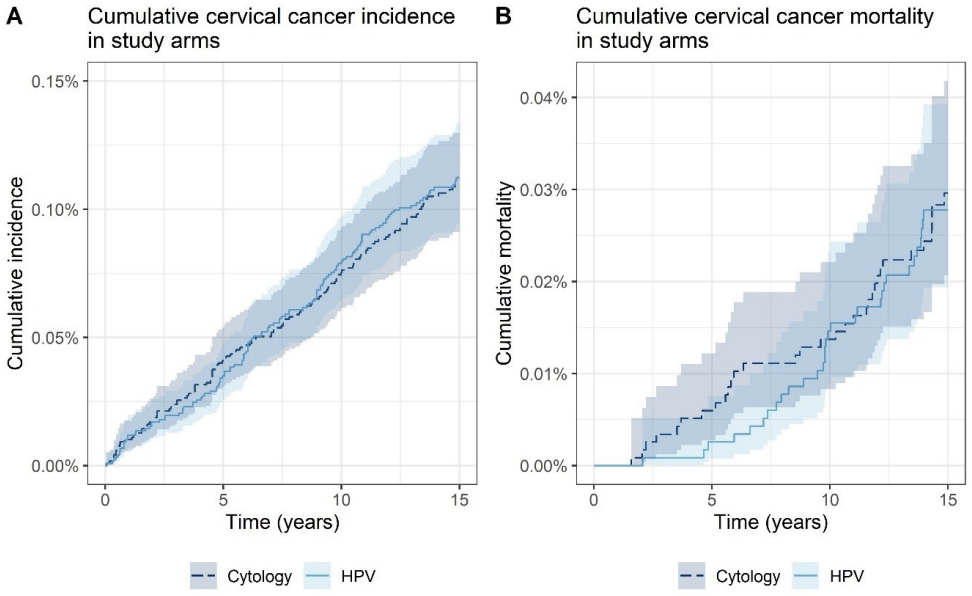


Figure 4 Cumulative incidence and mortality of cervical cancer in the trial study arms during a 15- year follow-up. The dashed dark blue line represents cytology screening arm, and the solid light blue line represents the HPV screening arm. The confidence intervals are shown as the less opaque areas around the incidence curves.

5.1.2 Long-term effectiveness on other anogenital cancers (II)

The study population in the Study II consisted of 118 217 individuals in the HPV arm, and 118 313 individuals in the cytology arm of the trial. Details of the inclusion criteria are presented in Study II, Figure 1.

During a follow-up of 1.74 million person-years in each study arm, we detected 20 vaginal cancers, and 60 vulvar cancers in the cytology arm, and 8 vaginal cancers and 44 vulvar cancers in the HPV arm. The IRR for vaginal cancer in the HPV arm was 0.40 (95% CI 0.17–0.88) compared to the cytology arm, and the corresponding IRR for vulvar cancer was 0.73 (95% CI 0.5–1.08), Figure 5. During the follow-up, we also detected 7 vaginal cancer deaths, and 11 vulvar cancer deaths in the cytology arm, and 5 vaginal cancer deaths, and 7 vulvar cancer deaths in the HPV arm. The MRR in the HPV arm compared to cytology arm was 0.72 (95% CI 0.21–2.24) for vaginal cancer and 0.64 (95% CI 0.23–1.62) for vulvar cancer.

Additionally, we assessed the number of anal cancers and anal cancer deaths during the follow-up. In the HPV arm, there was 32 anal cancers detected, and in the cytology arm there was 36 anal cancers detected. In both study arms, there were 4 anal cancer deaths. There was no significant difference in the anal cancer incidence (IRR 0.81; 95% CI 0.47–1.35), in the HPV arm compared to the cytology arm), or mortality (MRR 1.00; 95% CI 0.24–4.24).

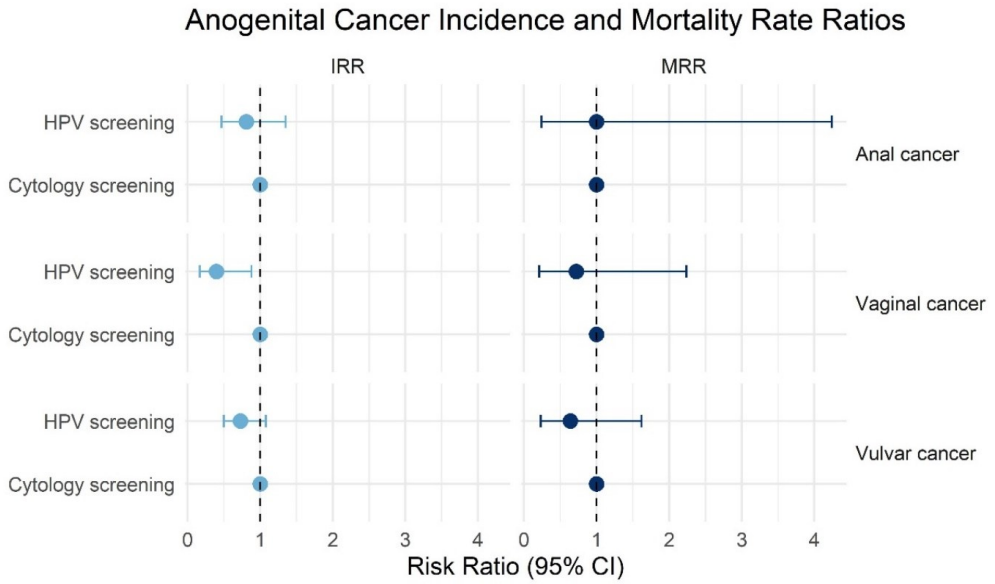


Figure 5 Incidence (IRR) and mortality (MRR) rate ratios for anogenital cancers in the study arms Cytology arm was the reference arm in the analyses. The dot represents the IRR and MRR estimates for each cancer and screening method and the bar represents the 95% confidence interval.

5.2 Triage of HPV-positives

5.2.1 Cytology

In this study we examined the performance of the five different HPV screening algorithms with cytology triage, one HPV screening algorithm without triage, and cytology screening algorithm presented in Chapter 4.3.2.1. We compared the episode sensitivities, episode specificities, and colposcopy referral rates these algorithms would have resulted in, if they would have been used in the first and second screening rounds of the HPV screening trial. The HPV Stand-alone algorithm, in which all HPV-positive individuals would have immediately been sent to colposcopy, resulted the highest HSIL+ sensitivity (93.6% in the first round, and 86.8% in the second), but the worst specificity (92.8% in the first round, and 95.2% in the second), and the highest colposcopy rate in both screening rounds, Figure 6 below. The specificity of this algorithm was, however, slightly improved in the second screening round. The Cytology screening algorithm, which was based on the routine cytology screening algorithm, had the highest specificity (99.0% in the first round, and 99.3% in the second), but the second lowest sensitivity in the first (68.6%) and in the second (66.7%) screening rounds.

Based on the analyses, the best balance between the episode sensitivity and episode specificity was with the algorithm HPV Persistence 2. In this algorithm, all HPV-positive cytology-negative individuals are followed up with two repeat tests before sending them to colposcopy. If the HPV infection persists in the second repeat test, the individual is sent to colposcopy, and if the infection is not detected anymore, the individual is sent back to the routine screening. This algorithm, however, had also twice as high colposcopy referral rate (3%) compared to the Cytology algorithm (1.5%).

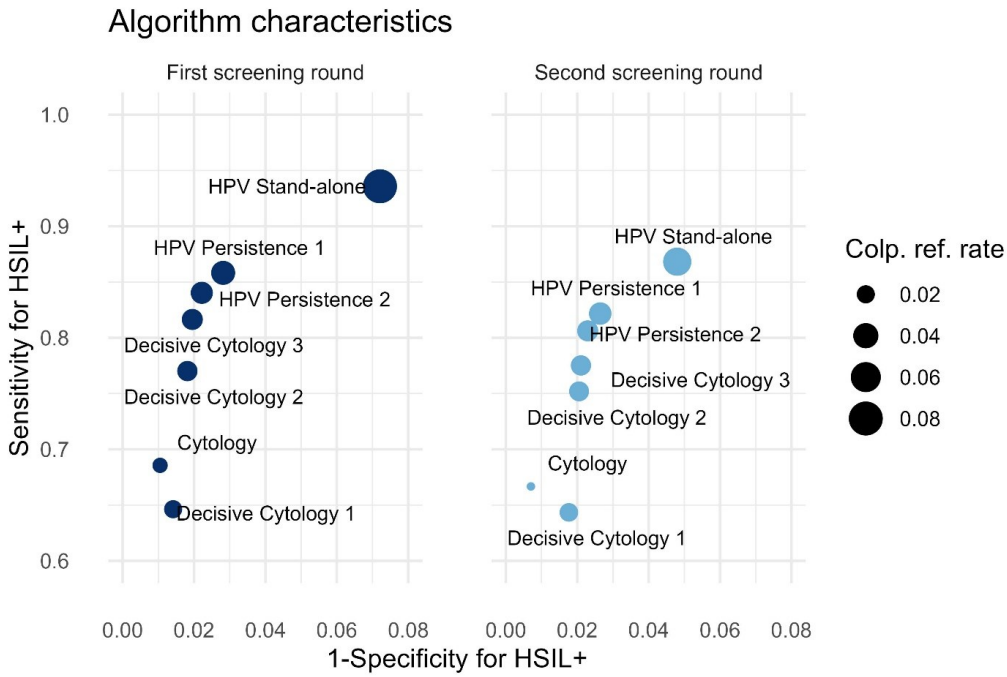


Figure 6 ROC-curve presenting the sensitivities and 1-specificities of each post-hoc screening algorithm for HSIL+. The size of the dot represents the colposcopy reference rate each algorithm evoked. The subgraph on the left shows the estimates for the first screening round of the trial and the right the estimates for the second screening round.

5.2.2 HPV genotyping

In the Study IV, we genotyped 5253 Hybrid Capture 2 positive HPV screening samples. Of these, 3695 (70.3%) were HPV-positive, and 1558 (29.7%) negative in the genotyping analyses. Multiple genotypes were present in 675/3695 (18.3%) of the positive samples.

Based on the genotyping analyses, HPV16 had the highest HSIL+ cumulative incidence (38.1%), followed by HPV33/58 (25.4%), and HPV31 (22.2%). The lowest cumulative incidences were observed for HPV56/59/66 (4.4%), HPV35/39/68 (6.5%), and HPV51 (7.5%). Individuals aged 50 or older at the entry test, had lower cumulative incidences for the highest-risk genotypes, HPV16 and HPV33/58, than the younger individuals. The age-specific cumulative incidences for all of the studied genotypes are presented in Study IV, Figure 4.

The cumulative incidence for HSIL+ with normal cytology at the baseline was the highest with HPV16 (25.3%; 95% CI 20.8%–30.0%), followed by HPV33/58 (16.9%; 95% CI 11.7%–22.8%), and HPV31 (15.9%; 95% CI 10.2%–22.7%), Figure 7 below. The lowest cumulative incidences with normal cytology were with HPV56/59/66 (4.1%; 1.6%-8.3%), and HPV51 (8.8%; 95% CI 4.6%-14.6%). HPV51 was the only genotype with no significant difference in the cumulative incidences between the LSIL+ and normal cytology triage results. Even with an LSIL+ cytology triage this genotype had a cumulative incidence of 7.8% (95% CI 2.5%–17.3%). Also, HPV56/59/66 had a low cumulative incidence with an LSIL+ cytology triage, 11.5% (95% CI 5.0%–21.0%).

For the highest risk HPV genotypes the differences in cumulative incidence between LSIL+ cytology and normal cytology were substantial. HPV16 had a 69% (95% CI 62.1%-74.7%) 18-year HSIL+ cumulative incidence with LSIL+ cytology, HPV31 a 60.0% (95% CI 46.3%-71.0%) cumulative incidence, and HPV33/58 a 41.0% (95% CI 31.2%-50.1%) cumulative incidence.

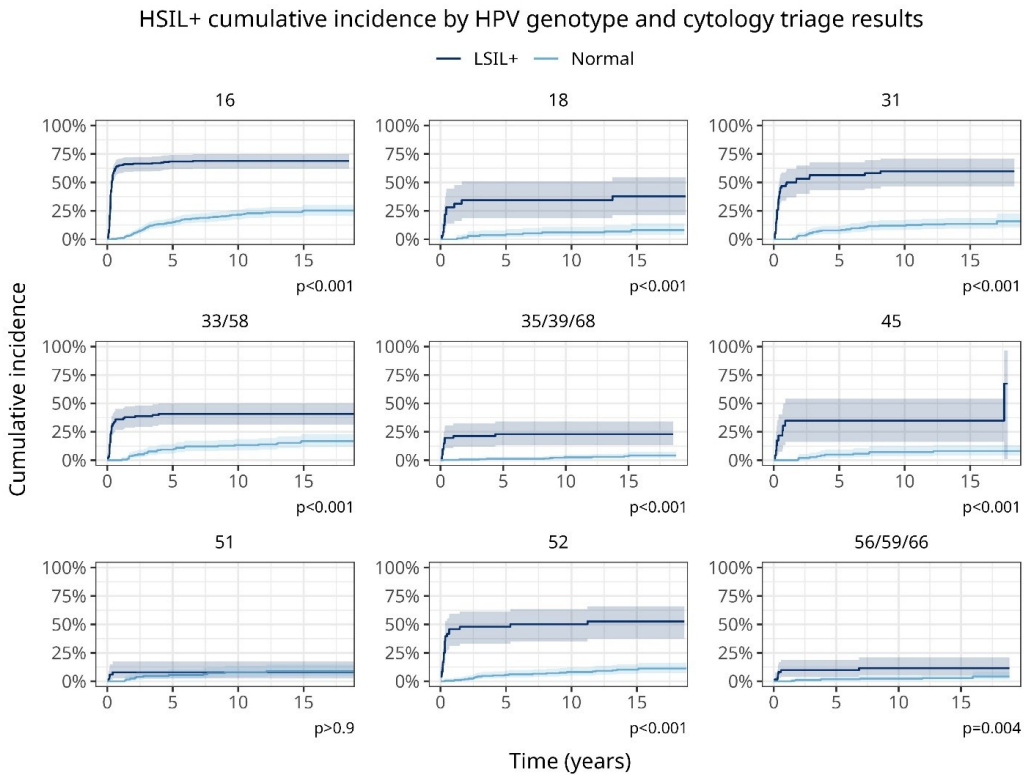


Figure 7 HSIL+ cumulative incidences by genotype and baseline cytology triage result. The cumulative incidence of normal cytology is shown as a light blue curve and the one of LSIL+ cytology as a dark blue curve. The p-values for the cytology-stratified cumulative incidence differences are shown below each subgraph.

6 Discussion

This thesis examined the benefits of HPV screening in comparison to cytology screening by evaluating the long-term effectiveness of HPV screening in the prevention of cervical cancer, as well as other anogenital HPV-related cancers. Furthermore, the thesis aimed to identify triage strategies, for individuals who test positive for HPV, enhancing the specificity of HPV screening. The findings are based on long-term follow-up data from one of the earliest HPV screening trials incorporated into routine screening programmes.

6.1 Long-term effectiveness of HPV screening

6.1.1 On cervical cancer

We found similar effectiveness with HPV screening than with cytology screening for cervical cancer incidence and mortality in the ITT analysis. Our results differ from previous results of a pooled study on European RCTs, which showed a 60–70% greater reduction in cervical cancer incidence in a 6.5-year follow-up. [9] Also observational studies have shown lower incidences of CIN3+ in the subsequent screening rounds of HPV screening programmes. [229,230]

We performed multiple sensitivity analyses and saw similar results as in the ITT analyses. In the sensitivity analysis restricted to the areas where the trial lasted for two rounds, the cumulative incidence and the cumulative mortality of cervical cancer were slightly, but not significantly, higher in the cytology arm than in the HPV arm.

The high frequency of testing outside the population-based screening programme in Finland [221,231], could play a role in our result. However, the number of tests conducted outside the organised screening programme was similar between the HPV and cytology study arms. To further evaluate the potential impact of these additional tests, we calculated the incidence and mortality rates among individuals who had no more than one test outside the organised programme during the trial period. Among this subgroup, cervical cancer incidence and mortality rates were also similar between the study arms. These findings suggest that our results are unlikely to be explained by testing conducted outside the screening programme.

The discrepancy between our results and other results possibly arises from the historically high performance of cytology screening in Finland [232], and from the low cervical cancer baseline risk among the Finnish population. [233] It is likely that in other types of settings HPV screening offers a better long-term protection against cervical cancer, even though we did not see this in our study.

Majority of individuals in our cohort were not screening naïve and continued with the routine cytology screening after the one or two trial screening rounds. The comparison between HPV and cytology screening could have been different in a screening naïve cohort. In a study with a previously unscreened high-risk cohort in rural India, a single round of HPV screening resulted in a significant reduction in advanced cervical cancers and cervical cancer deaths, unlike cytology screening. [234]

Improving screening effectiveness by using a more sensitive test can also be challenging in a setting similar to ours, because the majority of cervical cancer cases and deaths occur among individuals who are either beyond the eligible screening age or who do not participate in the screening programme. [235] A more effective way to further improve the screening effectiveness for cervical cancer could be targeted interventions to reach the non-attenders.

6.1.2 On other anogenital cancers

Although we did not observe greater effectiveness with HPV screening compared to cytology screening for cervical cancer, we did find a significantly lower incidence of vaginal cancer in the HPV screening arm. Specifically, there was a 60% reduction in vaginal cancer cases in the HPV group compared to the cytology group. Additionally, vaginal cancer mortality was nearly 30% lower in the HPV screening arm, although this difference was not statistically significant, likely due to the small number of deaths from vaginal cancer. Similarly, both the incidence (a reduction of nearly 30%) and mortality (a reduction of nearly 40%) of vulvar cancer were lower in the HPV arm compared to the cytology arm; however, these differences were likewise not statistically significant. We did not observe lower anal cancer incidence or mortality with cervical HPV screening than with cytology screening.

Our results suggest that HPV screening has a greater impact on the incidence of vaginal cancer than on that of vulvar cancer, which is likely due to the higher proportion of vaginal cancers attributable to HPV. Approximately 78% of vaginal cancers are HPV-related, compared to only around 25% of vulvar cancers. [117]

There are several possible options how cervical HPV screening could lower the incidence of other HPV-related genital cancers. Firstly, HPV test is more sensitive to detect CINs and these lesions are more likely to be treated when identified through HPV screening. This is particularly relevant in the context of the Finnish trial, where even CIN1 lesions were treated in the early years of trial. [173] Individuals with a cervical HPV infection have a higher risk of having an HPV

infection also in other anogenital sites. [236,237] Untreated cervical HPV infections may spread to these sites and cause anogenital cancers. However, evidence suggests that even when CIN lesions are treated, HPV can persist in the anogenital region. [238] Several studies have investigated the risk of subsequent malignancies following a diagnosis of cervical precancer or cancer [57,239], but none have been able to distinguish between the risks associated with untreated versus treated cervical lesions. Thus, further research is needed to validate this hypothesis.

Research has also shown a more rapid clearance of HPV infections after a cervical punch biopsy. [240] A higher colposcopy rate in the HPV screening arm could have led to a higher punch biopsy rate, and thus to more HPV infections being cleared.

Another possible explanation for how cervical HPV screening may reduce the incidence of other HPV-related genital cancers is the increased detection and treatment of HSILs of the vagina and vulva. Treatment of these precancerous lesions is recommended in order to reduce the risk of progression to invasive cancer. [241,242] To explore this hypothesis, we conducted a post hoc analysis of the number of vaginal and vulvar HSILs detected in each study arm. These results are presented in Study II. While the difference in the number of HSILs between the study arms was not statistically significant, there was a slightly higher number of vaginal HSIL diagnoses in the HPV screening arm compared to the cytology arm.

These findings are novel, as the effectiveness of cervical HPV screening in preventing other genital cancers has not been previously assessed. They suggest that in settings such as ours, where further gains in cervical cancer prevention through HPV screening are limited, the additional preventive effects on vaginal cancer, and potentially also on vulvar cancer, may provide a rationale for transitioning from cytology screening to HPV screening. This is particularly relevant given that decades of cytology screening in Finland and other Nordic countries have not decreased incidence of vaginal or vulvar cancers. [130]

However, this potential preventive effect requires confirmation through further studies before it can be used as an additional basis for decision-making. Moreover, the primary aim of cervical cancer screening is to reduce the morbidity and mortality associated with cervical cancer. Other genital cancers, which represent a considerably smaller public health burden, should not be the main determinant in decisions regarding cervical cancer screening strategies.

6.2 Optimal triage strategies

6.2.1 With cytology

The main harm of HPV screening is the higher rate of colposcopy referrals compared with cytology screening, due to its lower specificity. In Study III, our aim was to identify a cytology triage strategy that could reduce the referral rate whilst maintaining high screening sensitivity. We found that the optimal balance between sensitivity and specificity was achieved with an HPV algorithm involving two repeated follow-up tests for HPV-positive, cytology-negative individuals (HPV Persistence 2). The algorithm involving only one follow-up test (HPV Persistence 1) demonstrated a similar sensitivity but slightly lower specificity, resulting in a higher rate of colposcopy referrals compared to the approach with two follow-up tests. In contrast, Decisive Cytology Algorithms in which HPV-positive, cytology-negative individuals were returned to routine screening, either at the index test or after a single follow-up, showed higher specificity and lower colposcopy referral rates, but clearly had the lowest sensitivity for detecting HSIL+ lesions among all the HPV screening algorithms evaluated.

Of all the HPV screening algorithms, clearly the highest sensitivity was with HPV Stand-alone algorithm, in which all HPV-positive individuals were immediately sent to colposcopy. This algorithm, however, is not a feasible option as it resulted in the lowest specificity, and thus, created significantly higher colposcopy referral rates than the other HPV screening algorithms, especially the Cytology Algorithm.

Previous studies evaluating optimal algorithms for HPV screening with a cytology triage have reached varying conclusions and have focused on initial screening rounds. A Swedish study reported that the highest sensitivity was achieved using cytology triage followed by one follow-up test for individuals who tested positive for HPV, however, this study also incorporated partial genotyping in the follow-up testing. [243] In contrast, a Dutch study identified an algorithm similar to the Decisive Cytology Algorithm 2 as the most feasible option, primarily due to the increased colposcopy rates associated with alternative algorithms. [244] This suggests that there might not be a universal approach to HPV screening with cytology triage, as healthcare systems vary across countries, particularly in terms of their capacity to manage colposcopy volumes. Additionally, other factors, such as the prevalence of HPV, rates of screening attendance, and the baseline risk of cervical cancer, may influence the performance and suitability of different screening algorithms.

All HPV screening algorithms with cytology triage, however, resulted into an increase in colposcopy referrals compared to the Cytology Algorithm. Referral rates were higher for HPV screening algorithms not only in the first screening round but

also remained elevated during the second screening round, in comparison to the Cytology Algorithm. Notably, the colposcopy referral rate within the Cytology Algorithm also decreased in the second round, likely due to the cohort being five years older by that time, as the youngest age groups tend to have a higher prevalence of abnormal findings. [245,246] Thus, part of the observed decrease in referral rates within the HPV screening algorithms may also be attributable to the ageing of the cohort.

An additional noteworthy finding is that, although the HPV screening algorithm used in the trial (HPV Persistence 2) demonstrated greater sensitivity for detecting HSIL+ lesions compared to the Cytology Algorithm, this did not translate into a reduction in cervical cancer incidence or mortality in the effectiveness analyses. This suggests the potential overdiagnosis of cervical lesions, a concern that had already been suspected during earlier stages of the trial. [213]

Nevertheless, the overarching conclusion from the analysis of these algorithm characteristics is that it is difficult to optimise both the sensitivity and specificity of HPV screening solely with cytology triage.

6.2.2 With HPV genotyping

Based on our findings in Study IV, the incorporation of HPV genotyping into screening algorithms may offer a potential solution to this challenge. Given that knowledge regarding long-term, genotype-specific risks remains limited, particularly stratified with triage cytology, we calculated these risks to provide further insight into the potential utility of HPV genotyping in conjunction with cytology triage within HPV screening programmes.

In this study, we identified four HPV genotype risk groups for HSIL+. HPV16 was associated with a risk exceeding 30%, while HPV33/58 and HPV31 were linked to risks above 20%. HPV52, HPV18, and HPV45 showed risks greater than 10%, whereas HPV51, HPV35/39/68, and HPV56/59/66 were associated with risks below 10%. These findings are broadly consistent with those of previous studies assessing long-term genotype-specific risks. DeMarco et al. reported that HPV16 carried a uniquely high risk for CIN3+ compared to other genotypes and proposed categorising the remaining types into three groups based on their seven-year cumulative risk: HPV18 and 45; HPV31, 33, 35, 52, and 58; and HPV39, 51, 56, 59, and 68. [18] Similarly, a study by Smelov et al. examined 14-year cumulative incidences for CIN3+ and grouped genotypes accordingly: HPV16, 18, 31, and 33 with risks exceeding 28%; HPV35, 45, 52, and 58 with risks between 14% and 18%; and HPV39, 51, 56, 59, 66, and 68 with risks below 10%. [185]

Similar to our study, both DeMarco et al. and Smelov et al. reported a higher cumulative risk associated with HPV33 than with HPV18. However, DeMarco et al. classified HPV18 and HPV45 as constituting the second highest risk group, due to

their stronger association with cervical cancer, particularly adenocarcinoma, which may not be adequately captured in studies focusing on cervical precancer outcomes and with intermediate-length follow-up. Our study included a follow-up period of up to 18 years, and still, we did not observe an increased cumulative risk associated with HPV18. It is important to note, however, that our primary outcome measure was HSIL+, which is associated with an even higher precancer-to-cancer ratio than CIN3+. Consequently, we conducted a post hoc analysis to identify the number of cervical cancer cases among individuals with an HPV18 infection during follow-up and found none.

A separate Finnish study, based on an independent sample set, similarly reported a lower prevalence of HPV18 in HSIL+ lesions compared with HPV16, HPV31, and HPV52. [87] As outlined in the introduction, different variants of HPV16 and HPV18, with varying carcinogenic potential, have been identified, and there is evidence suggesting geographical variation in the distribution of these variants. [37,247] Thus, we cannot exclude the possibility that the predominant HPV18 variant prevalent in Finland differs from those found in other populations. Differences in the prevalence of cofactors affecting cervical cancer development, such as HIV infection, could also influence both the prevalence and risk associated with each HPV genotype. [248]

Our analyses also included genotype-specific risk estimates by age and cytology triage result. In the genotype- and age-specific analyses only significant finding was that with the highest risk genotypes, HPV16 and HPV33/58, cumulative incidence was lower among individuals aged 50 or over at entry, compared to younger individuals. For most of the genotypes there was no risk difference between the age groups, which is consistent to findings of a previous study. [18]

In contrast, cytology-stratification provided a wider range of risk estimates than age-stratification. For HPV genotypes associated with the highest risk of HSIL+ (HPV16, HPV33/58, and HPV31) a normal cytology result did not indicate low risk, with HSIL+ rates exceeding 15%. In comparison, for HPV51 and HPV56/59/66, the HSIL+ risk remained relatively low even when cytology resulted in LSIL or more severe findings. A systematic review by Bonde et al., which assessed the evidence for CIN3+ risk discrimination using HPV genotyping, also concluded that combining cytology triage with HPV genotyping could improve risk stratification. The systematic review further found that, based on available evidence with up to three years of follow-up, HPV35, 39, 51, 56, 59, 66, and 68 represent such a low precancer risk, even with LSIL+ cytology, that they could be followed up with a repeat test rather than referred directly to colposcopy. Our findings, based on a significantly longer follow-up period, further support this approach, specifically for the genotypes 51, 56, 59, and 66, as the risks for these genotypes remained low throughout the follow-up. Based on our results, it may also be appropriate to refer

individuals with the highest-risk genotypes (HPV16, 31, 33/58) to colposcopy even if they have a normal cytology.

6.3 Strengths and weaknesses

The main strength of this thesis is the high-quality individual-level data from several population-based nationwide registries. The Screening Register has data on nearly all of the screening invitations and tests taken in the cervical cancer screening programme. [249] The Finnish Cancer Register has an estimated completeness of 96% for solid malignant tumours. [250] Using personal identifiers, we were able to link data from the Finnish Cancer Register, the Screening Register, the Care Register for Health Care, Statistics Finland, records of tests performed outside the screening programme, and HPV genotyping results. These comprehensive data also enabled the conduct of multiple sensitivity analyses.

Furthermore, the randomised design of our study data enhances the reliability of the results. In Studies I and II, we presented the baseline characteristics of both randomised screening arms and demonstrated that they were comparable across all studied variables. Access to data from several different registries allowed us to assess the similarity across various sociodemographic factors, screening histories, and the number of tests conducted outside the screening programme. This supports the conclusion that the randomisation was successful and helped to control for both known and unknown confounding factors.

A major strength of our study was the inclusion of cervical precancers detected outside the population-based screening programme, obtained from the Care Register for Health Care. Including such cases allowed us to assess the characteristics of the post hoc screening algorithm retrospectively in Study III, and enabled us to obtain adequate genotype-specific risk estimates in Study IV.

In Study IV, another strength was that all genotyped screening samples were collected as part of a trial embedded within the population-based screening programme, ensuring that the cohort of HPV-positive individuals accurately represented actual screening participants.

A major limitation of our study design was that the largest municipality in Finland, Helsinki, decided to withdraw from the trial after the first screening round and continued with routine cytology screening. As a result, only 43% of individuals invited to the trial in the first round were also invited to the second screening round. However, in Studies I and II, we conducted sensitivity analyses restricted to the areas where the trial continued for two screening rounds. These analyses produced results consistent with the ITT analyses for all individuals invited in the first round.

Another limitation concerns the HPV test used in the trial, HC2, which is no longer commonly used in HPV screening. Nevertheless, HC2 remains a comparator

assay for the validation of new tests, and most currently approved tests perform similarly to it. [183]

In Studies III and IV, a further limitation was our inability to use CIN3+ as the outcome, as the data from the Screening Register we had classified histological HSIL cases without distinguishing between CIN2 and CIN3 lesions. This is a significant shortcoming, as CIN2 lesions are more likely to regress spontaneously and, in some cases, may represent overdiagnosis rather than early detection. Nevertheless, screening programmes still aim to detect HSIL+ lesions, and treatment of persistent CIN2 is recommended in most cases to prevent progression. [251] Thus assessing the sensitivities of different algorithms to HSIL+ and assessing the genotype-specific risks for HSIL+ also give important information for the screening programme organisers.

Finally, in Study IV, the use of two different genotyping assays was a limitation. Although these assays show high concordance in detecting high-risk HPV genotypes, minor discrepancies have been reported. [252] As a sensitivity analysis, we assessed HSIL+ risks among genotype-negative individuals separately for the two batches analysed with different assays. The risks were comparable, suggesting similar performance between the two assays.

It is also notable that our trial cohort consisted entirely of unvaccinated individuals, which limits the generalisability of the findings to vaccinated populations.

6.4 Future implications

Although we did not observe greater effectiveness in preventing cervical cancer with HPV screening, its comparable effectiveness, combined with the many other advantages it offers over cytology, makes it the preferred option for primary cervical screening programmes. One of these advantages is the possibility of HPV self-sampling. As discussed in Chapter 6.1.1, an effective way to further enhance cervical cancer screening could be through targeted interventions aimed at populations with low screening uptake. HPV self-sampling provides a practical tool for this purpose, and an important next step would be to implement HPV self-sampling pilot programmes in various underscreened populations and evaluate the impact of these interventions on cervical cancer incidence and mortality. A recent nationwide study from Sweden successfully detected a high number of HSIL+ cases by personally inviting women at high risk of cervical cancer to order HPV self-sampling kits. [253] A pilot study offering self-sampling to non-attendees and evaluating the self-sampling preferences among women has also recently started in Finland. [254]

In this thesis, we identified a potential novel benefit of HPV screening, as a lower incidence of vaginal cancer was observed in the HPV screening group compared to the cytology group. Given the rarity of vaginal cancer, population-wide

screening is not feasible; therefore, achieving a reduced incidence as a secondary benefit of cervical cancer screening would be highly advantageous. However, this finding requires validation through further studies and additional research into the underlying mechanisms. It also provides an important indication that high-risk populations, such as individuals living with HIV, might benefit from targeted vaginal cancer screening using HPV tests. [255]

Our results showed that the harms of HPV screening are difficult to tackle with cytology triage of HPV-positive individuals alone. However, adding HPV genotype information to the screening algorithm is likely to lead to better risk stratification in screening programmes. Our findings highlight the importance of extended genotyping, rather than partial genotyping where only HPV16 and HPV18 are distinguished from the other high-risk HPV types. We also showed that combining cytology triage results with genotype information provides additional insight into HSIL+ risk in screening programmes.

To keep screening algorithms manageable, it is helpful to group genotypes according to their risk level. Based on our results, the highest risk group includes HPV16, 31, and 33/58, with infections possibly needing referral to colposcopy, even with a normal cytology result. The intermediate risk group comprises HPV18, 45, 52, and 35/39/68. For individuals in this group, cytology triage provides valuable additional risk stratification: those with LSIL+ cytology could be referred to colposcopy, while those with ASCUS or NILM cytology could be retested after one year. Individuals infected with a type with a lower risk (HPV51, 56/59/66) and with NILM cytology could return to routine screening, while those with cytological abnormalities should be retested after 12 months. However, the performance of such an approach should be piloted before large-scale implementation. Incorporating other risk-stratifying factors, such as previous screening history, has also been shown to be beneficial and could further support the categorisation of screening participants into appropriate risk groups. [253]

Furthermore, cervical cancer screening is expected to undergo significant changes in the coming decades as vaccinated individuals begin to enter screening programmes. How screening should be organised for these vaccinated cohorts remains uncertain and represents an important research question for future studies. Vaccination will also reduce the incidence of other anogenital cancers, making it essential to consider vaccine impact in future research evaluating the effectiveness of screening methods for HPV-related cancers. As vaccination coverage increases, research into and the implementation of risk stratification within HPV screening programmes becomes even more critical to ensure strategies that maximise benefits and minimise harms.

7 Conclusions

1. While HPV screening may not significantly improve the effectiveness of cervical cancer detection in settings similar to ours, with a well-established cytology programme and a low baseline risk for cervical cancer, it remains a feasible primary screening method due to its additional advantages, such as objectiveness and the possibility of self-sampling.

2. Cervical HPV screening may offer additional benefits in preventing other HPV-related genital cancers. However, the exact mechanisms underlying this preventative effect require further investigation.

3. The optimal balance between sensitivity and specificity for detecting HSIL+ in HPV screening with cytology triage was achieved using an algorithm in which HPV-positive but cytology-negative individuals underwent two follow-up tests. Referral to colposcopy occurred if the HPV infection persisted in both follow-ups or if cytological abnormalities developed. However, this algorithm still resulted in high colposcopy referral rates during the first and second screening rounds. Therefore, cytology triage alone is insufficient to mitigate the increased referral rates associated with primary HPV screening.

4. Incorporating extended genotyping into HPV screening algorithms with cytology triage offers the potential to reduce colposcopy referral rates through more accurate risk stratification. High-risk genotypes (HPV16, 31, 33/58) were associated with elevated HSIL+ risks even with normal cytology, whereas some genotypes (HPV51, 56/59/66) demonstrated low HSIL+ risks even when cytology indicated LSIL or worse. Screening algorithms utilising this information on genotype-specific risks should be piloted within existing screening programmes.

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