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To my daughter Elizabeth

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Olga Veijalainen

ABSTRACT

Thanks to an organized system of cytology-based cervical cancer screenings, the incidence and mortality from cervical cancer in Finland has declined significantly since the 1960s. The acknowledgement of the strong causal role of high-risk human papillomavirus (hrHPV) infections in the pathogenesis of cervical cancer and its precursors has led to the development of testing for oncogenic hrHPV types. HrHPV tests have been successfully studied in triage of minor cytological cervical lesions, in the follow-up after treatment of high-grade cervical lesions and, finally, as a new tool for primary screening of cervical cancer. The change from conventional cytology to HPV-based screening in the population-based cervical screening program is perhaps the biggest single change in the screening program for cervical cancer in Finland

The primary aim of the current study was to evaluate the performance of implementing primary HPV screening in the detection of precancerous cervical lesions within the regional routine organized cervical screening program in the city of Tampere. The Abbott RealTime (ART) hrHPV DNA test was used as a cervical cancer screening test.

The last totally conventional cytological screening took place in 2011, and the following year, the primary hrHPV cervical screening program was implemented. The results of the first screening round demonstrated that the introduction of the primary HPV screening did not affect the pattern of attendance between the cytology-based and hrHPV-screened groups. The attendance rate in the last round of cytological screening and in the first round of HPV screening were equal and corresponded to the level of attendance of cervical cancer screenings in Finland (approximately 70%).

In the first year of implementation of primary HPV screening the detection rate of cervical intraepithelial neoplasia grade two or worse (CIN2+) doubled in the HPV-screened population. The detection rate of CIN3+ lesions was also higher in the HPV-screened population, but the difference was not statistically significant.

The first three years of experience confirmed a higher detection rate of both CIN2+ and CIN3+ lesions in comparison with conventional cytology (the Relative Risk (RR) 2.45 (95%CI 1.76-3.41) for CIN2+ and RR 2.70 (95%CI 1.75-4.16) for CIN3+.

During the first years of implementation of HPV screening, the policy to refer all women with persistent HPV infections for colposcopy resulted in a doubling of the rate

of colposcopy among HPV-screened women in comparison with cytological screening. However, in the second screening round, the colposcopy rate decreased significantly, by 29%.

The outcome from the second screening round demonstrated a 27% (p = 0.220) decrease of histological HSIL+ lesions, the result was not statistically significant. Nevertheless, the available evidence from randomized controlled trials shows the benefit of primary HPV screening in the reduction of CIN3+ lesions in subsequent screening rounds and the following reduction of morbidity and mortality from cervical cancer.

The high detection rate of potentially non-progressive CIN2+ lesions in our studies led to a high rate of follow-up tests and colposcopies, which risked overdiagnosis and overtreatment. A reliable triage test for HPV-positive women and an optimal screening protocol for women with persistent HPV infections are obviously needed to help avoid the harms of primary HPV screening and to refer for follow-up studies and treatment only women with lesions that have a high cancer potential.

The second aim of the study was to evaluate the real-life performance of hrHPV tests in the triage of women with low-grade squamous intraepithelial lesions (LSIL) and repeat atypical squamous cells of undetermined significance (ASC-US). HrHPV testing turned out to be beneficial in the triage of repeat ASC-US in women ≥30 years old. However, in the case of LSIL, the prevalence of hrHPV infections was high (around 70%), and the specificity of the hrHPV tests used (Hybrid Capture II and ART hrHPV DNA tests) was low (36%), limiting the usefulness of hrHPV testing in triage of LSIL.

TIIVISTELMÄ

Valtakunnallisen sytologisen kohdunkaulasyövän seulonnan ansioista syövän ilmaantuvuus ja kuolleisuus ovat merkitsevästi laskeneet 1960-luvulta lähtien. Korkean riskin papilloomaviruksen (hrHPV) keskeinen kausatiivinen rooli kohdunkaulan syövän ja sen esiasteiden patogeneesissä on johtanut hrHPV-testien kehittämiseen. HrHPV-testien käyttökelpoisuutta on menestyksekkäästi tutkittu kohdunkaulan lievien solumuutosten seurannassa, vahvojen solumuutosten hoitotulosten seurannassa ja kohdunkaulasyövän primaariseulonnassa. Siirtyminen sytologisesta seulonnasta HPV-seulontaan väestöpohjaisessa kohdunkaulan syövän seulontaohjelmassa on kenties suurin yksittäinen muutos meidän maamme organisoidussa väestöpohjaisessa kohdunkaulan syövän seulontaohjelmassa.

Tämän väitöskirjan ensimmäisenä tavoitteena oli selvittää primaarisen HPV-seulonnan toteuttavuutta ja löydöksiä Tampereen kaupungin organisoidun väestöpohjaisen kohdunkaulasyövän seulontaohjelman puitteessa. Seulontavälineenä oli Abbottin RealTime (ART) hrHPV-DNA-testi ja varmistusmenetelmänä (triage) sytologinen (Papa) testi.

Viimeinen sytologinen seulonta toteutettiin vuonna 2011, ja seuraavana vuonna HPV-seulonta otettiin käyttöön. Ensimmäinen seulontakierros osoitti, että uusi seulontamenetelmä ei vähentänyt seulontaan osallistumista. Sekä vuoden 2011 että vuoden 2012 seulontojen osallistumisaktiivisuudet vastasivat yleistä Suomen kohdunkaulan syövän seulontoihin osallistuvuutta (noin 70%).

Ensimmäisellä HPV-seulontakierroksella todettiin kaksinkertainen määrä vähintään kohtalaisia (CIN2+) muutoksia HPV-ryhmässä verrattuna sytologiseen seulontaan. Myös vähintään vaikea-asteisia (CIN3+) muutoksia todettiin lukumääräisesti enemmän HPV-ryhmässä, mutta ero ei ollut merkitsevä.

Kolmen ensimmäisen vuoden HPV-seulonnan tulokset osoittivat uuden seulontamenetelmän selvän edun sekä CIN2+ että CIN3+ -tasoisten muutosten havaitsemisessa sytologiseen testiin verrattuna. HPV-ryhmässä löytyi 2,45 kertaa enemmän CIN2+ -muutoksia (95% luottamusväli 1,76–3,41) ja 2,70 kertaa enemmän CIN3+ -muutoksia (95% luottamusväli 1,75–4,16).

Ensimmäisillä HPV-seulontakierroksilla kolposkopioiden määrä kaksinkertaistui HPV-ryhmässä sytologiseen seulontaan verrattuna, koska kaikki naiset, joilla oli

persistoiva hrHPV-infektio, ohjattiin kolposkopiaan riippumatta Papa-testin tuloksesta. Vuoden 2017 seulontakierroksella suoritettujen kolposkopioiden määrä oli kuitenkin laskenut merkitsevästi (29%) kohortissa, joka oli seulottu kahdesti hrHPV-testillä.

Toisella seulontakierroksella tapahtunut histologisten HSIL+ -muutosten (CIN2+ ja CIN3+) väheneminen (27 %) ei ollut tilastollisesti merkitsevä (p=0.220). Toisaalta satunnaistetuista tutkimuksista saatu näyttö on selvästi osoittanut, että HPV-seulonnan ansiosta CIN3+ -muutokset vähenevät tilastollisesti merkitsevästi toisella seulontakierroksella ja tämän seurauksena sekä sairastuvuus että kuolleisuus kohdunkaulasyöpään laskevat.

Potentiaalisesti etenemättömien CIN 2+ -muutosten huomattava määrä johti tässä tutkimuksessa suureen määrään kontrollitestejä ja tarpeettomia kolposkopioita, ja edelleen ylidiagnostiikan ja ylihoidon riskiin. Tämä voitaisiin välttää HPV-positiivisten naisten optimaalisella tarkistustestillä (triage), jolloin tunnistettaisiin ne naiset, joiden kohdunkaulasyövän riski on todella kohonnut ja lähettämään jatkotutkimuksiin ja hoitoon vain heidät.

Väitöskirjan toisena tavoitteena oli arvioida hrHPV-testin soveltuvuutta varmistustestinä naisilla, joilla on Papa-testissä lieviä levyepiteelimuutoksia (LSIL) tai toistuvia epäspesifisiä solumuutoksia (ASC-US). HrHPV-testi osoittautui soveltuvaksi yli 30-vuotiaiden naisten toistuvien ASC-US-muutoksien varmistustestinä. Korkea HPV infektioiden esiintyvyys (noin 70%) ja matala käytettyjen hrHPV-testien (Hybrid Capture II ja Abbott RealTime) spesifisyys (36%) kuitenkin rajoittavat hrHPV-testin käyttökelpoisuutta LSIL -muutosten varmistustestinä.

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ABBREVIATIONS AND SYMBOLS

AC	Endocervical adenocarcinoma
AGC	Atypical glandular cell
AGC+	Atypical glandular cell or more severe lesion
AIS	Adenocarcinoma in situ
ALTS	The ASCUS-LSIL Triage Study
ART	Abbott RealTime
ASC-H	Atypical squamous cells, cannot exclude HSIL
ASC-US	Atypical squamous cells of undetermined significance
ASR	Age-standardised rate
CC	Conventional cytology
CI	Confidence interval
CIA	Confidence Intervals Analysis
CIN	Cervical intraepithelial lesions
CIN1	Cervical intraepithelial lesions grade 1
CIN2	Cervical intraepithelial lesions grade 2
CIN3	Cervical intraepithelial lesions grade 3
CIN1+	Cervical intraepithelial lesions grade 1 or more severe lesion
CIN2+	Cervical intraepithelial lesions grade 2 or more severe lesion
CIN3+	Cervical intraepithelial lesions grade 2 or more severe lesion
CIS	Carcinoma in situ
DNA	Deoxyribonucleic acid
DS	Dual-stained cytology
ECC	Endocervical curettage
GLOBOCAN	An online database providing global cancer statistics and estimates of incidence and mortality in 185 countries for 36 types of cancer
HC2	Hybrid Capture 2
HDI	Human Development Index
HIV	Human immunodeficiency virus
HrHPV	High risk human papillomavirus
HPV	Human papillomavirus
HPVA	HPV-associated endocervical adenocarcinoma

HR	Hazard ratio
HSIL	High grade squamous intraepithelial lesions
HSIL+	High grade squamous intraepithelial lesions or more severe lesion
IARC	International Agency for Research on Cancer
ICC	Invasive cervical cancer
IECC	The International Endocervical Adenocarcinoma Criteria and Classification
LBC	Liquid-based cytology
LEEP	Loop electrosurgical excision procedure
LLEZ	Large loop excision of the transformation zone
LrHPV	Low risk human papillomavirus
LSIL	Low-grade squamous intraepithelial lesions
NHPVA	HPV-unassociated endocervical adenocarcinoma
NILM	Negative for intraepithelial lesion
NORDCAN	A database of cancer statistics for the Nordic countries: Denmark, Finland, Iceland, Norway, Sweden, Faroe Islands and Greenland
NOS	Not otherwise specified
NPV	Negative predictive value
NTCC	The New Technologies in Cervical Cancer
PPV	Positive predictive value
OR	Odds ratio
PAP	Papanicolaou
PCR	Polymerase chain reaction
RCT	Randomized controlled trial
RR	Relative risk
SCJ	Squamocolumnar junction
SCC	Squamous cell carcinoma
TBS	The Bethesda System
VALCENT	Validation of HPV genotyping test
VIA	Visual inspection of the cervix with acetic acid
WHO	World Health Organisation

LIST OF ORIGINAL PUBLICATIONS

I   Veijalainen O, Tuomisaari S, Luukkaala T, Mäenpää J. High risk HPV testing in the triage of repeat ASC-US and LSIL. Acta Obstet Gynecol Scand 2015; 94: 931-6.

II   Veijalainen O, Kares S, Kujala P, Tirkkonen M, Vuento R, Kholová I, Luukkaala T, Osuala V, Mäenpää J. Human papillomavirus test with cytology triage in organized screening for cervical cancer. Acta Obstet Gynecol Scand 2016; 95: 1220-7.

III   Veijalainen O, Kares S, Kujala P, Vuento R, Osuala V, Tirkkonen M, Luukkaala T, Kholová I, Mäenpää J. Implementation of HPV-based cervical cancer screening in an organised regional screening programme: 3 years of experience. Cytopathology 2019; 30:150-156.

IV   Veijalainen O*, Kares S*, Kotaniemi-Talonen L, Kujala P, Vuento R, Luukkaala T, Kholová I, Mäenpää J. Primary HPV screening for cervical cancer: Results after two screening rounds in a regional screening program in Finland. Acta Obstet Gynecol Scand. 2021; 100: 403-9.

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1 INTRODUCTION

Our knowledge of the etiology, pathogenesis, detection, prevention, and treatment of cervical cancer has developed rapidly in the last century. Now, we can even propose the elimination of cervical cancer as a public health problem.

Dr. George Papanicolaou, in collaboration with gynecological pathologist Dr. Herbert Traut, published *Diagnosis of Uterine Cancer by the Vaginal Pap Smear* in 1943. The book contains descriptions and illustrations of normal and abnormal vaginal and cervical cells. Soon after, the Pap smear became the gold standard screening test for cervical cancer. Since the middle of the last century, the incidence and mortality from cervical cancer over the past years has significantly decreased in many developed countries after the implementation of the population-based cervical cytology screening programs. It has been estimated that in the Nordic countries of Denmark, Norway, Finland and Sweden, the incidence rate of cervical cancer in 2006–2010 would have been between three and five times higher without screening. Over 60,000 cases, or between 41% and 49% of the expected infection rate, were prevented by the introduction of cytological-based cervical cancer screening in the late 1960s and early 1970s (Vaccarella et al. 2014). In Finland, the implementation of the national cervical cancer screening program led to an 80% reduction in incidence of cervical cancer from 15/100,000 woman-years in 1963 to 4/100,000 in 2007 (Kotaniemi-Talonen et al. 2007; Van Der Aa et al. 2008). According to the Finnish Cancer Registry, the annual number of new cervical cancer cases is currently about 160, and the participation rate is about 70% (191,000/272,000 invited women in 2018). The effect of cervical cancer screening in Finland between 2000 and 2009 on the risk and mortality from cervical cancer has been demonstrated in a case-control evaluation of the Finnish program. Screening participation was associated with a 47% reduction in the risk of cervical cancer and a 66% reduction in the risk of mortality from cervical cancer (Lönnberg, Anttila, et al. 2012; Lönnberg et al. 2013). The audit of cervical cancers between 2000 and 2009 demonstrated a clearly higher proportion of the advanced disease in non-invited women (56.2%) compared with screening attenders (14.5%) and nonattenders (29.3%) (Lönnberg, Anttila, et al. 2012). Among the 506 cervical cancer deaths between 2000 and 2009, 60.7% were

registered in women non-invited to screening, 23.9% were registered in non-attenders, and 15.4% were registered in screening attenders (Lönnberg et al. 2013).

Cytological-based screening is considered to have a high specificity for high-grade cervical intraepithelial lesions (96%–98%). However, Pap cytology has several deficiencies, the main one being its relatively low sensitivity (51%–53%) (Nanda et al. 2000; Cuzick et al. 2006). Between 1990-1999, 23% of cervical cancers were diagnosed in women with normal screening test results, meaning that about 30–40 women with normal screening tests had cervical cancer each year (Lönnberg et al. 2010). This demonstrated the need for a more effective test for need for a more effective test for women participating in the screening program.

The limitations of cytological screening have led to the conclusion that Pap screening's maximum impact on global cervical cancer prevention has been reached (Kitchener, Castle, and Cox 2006). Nevertheless, cytological screening is the primary screening test for cervical cancer in many countries.

The causal role of persistent high-risk papilloma virus (hrHPV) infection in the development of cervical cancer has opened a new opportunity for clinical application of HPV testing in triage of women with equivocal or low-grade cytological lesions, in prediction of the results after treatment of high-grade cervical intraepithelial neoplasia (CIN), in primary screening of cervical cancer and precancer (Hausen 1976; Bosch et al. 1995; Walboomers et al. 1999; Arbyn et al. 2012).

Within the current decade, many low- and high-resource countries have adopted primary HPV testing for primary cervical cancer screening. HPV testing has a greater sensitivity for detecting high-grade precancerous disease and has demonstrated better protection for cervical cancer with a lower risk of developing cervical cancer or precancer after a negative HPV test in comparison to a negative Pap smear (Dillner et al. 2008; Wright et al. 2015; Koliopoulos et al. 2017).

Since the 1960s, cytology-based screening has been implemented in Finland through an organized program for all women aged 30 to 60 years with a five-year screening interval. Currently, some municipalities offer cervical screening for women as young as 25 and/or as old as 65. In Finland, primary HPV screening was initiated as a randomized trial within the national organized cervical cancer screening program in 2003 (Kotaniemi-Talonen et al. 2005). Primary HPV screening with Pap triage replaced cytology screening in the city of Tampere on 1 January 2012.

The purpose of the current study was to investigate the benefits and harms of the implementation of primary HPV screening within a regional population-based cervical cancer screening program in the city of Tampere. The secondary aim was to

assess the use of the HPV test in the management of women with ASC-US and LSIL cytology.

2 REVIEW OF THE LITERATURE

2.1 Classification of tumours of the uterine cervix

Cervical cancer and its precursors develop in the cervix, the fibromuscular lower part of the uterus that connects to the vagina. This organ is covered with two different types of epithelia: the ectocervix is lined by a stratified squamous epithelium and the endocervix is lined by a simple columnar epithelium. The transition area where the squamous epithelium and columnar epithelium meet is called the squamocolumnar junction (SCJ). Approximately 90% of cervical cancer and its precursors develop in the transformation zone, the region between the original SCJ and the new SCJ (Burghardt and Ostor 1983).

Over the past 120 years, numerous histopathological classifications have been used. In 1886, Sir John Williams discovered a noninvasive abnormal epithelium in specimens from invasive squamous cell carcinomas (*Blaustein's Pathology of the Female Genital Tract* 2011). In 1952, Reagan and Hicks proposed a classification of precancerous lesions including four levels: mild dysplasia, moderate dysplasia, severe dysplasia and carcinoma in situ (Reagan and Hicks 1953). In 1969, Richard and Barron proposed the three-tiered cervical histopathological terminology: cervical intraepithelial neoplasia 1 (CIN1), which includes mild dysplasia and condyloma (anogenital warts); CIN2, which includes moderate dysplasia; and CIN3, which includes both severe dysplasia and carcinoma in situ (CIS) (Richart and Barron 1969).

Improved knowledge of HPV biology and pathogenesis as well as a new classification of cytology with the Bethesda System led to a modification of the three-tiered classification (Nayar and Wilbur 2015). In 2012, the Lower Anogenital Squamous Terminology Project approved a two-tiered classification system of histopathological terminology for squamous intraepithelial lesions: low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL) (Darragh et al. 2012). The World Health Organization (WHO) introduced this classification in 2014 (Kurman et al. 2014). The new terminology reflected two general conditions developed after HPV infections in squamous epithelia: LSIL with a low risk for progression and transit HPV infection and HSIL

with a high risk for progression and a substantial risk for malignancy (Darragh et al. 2012) (Table 1). LSIL include CIN1, mild dysplasia and koilocytotic atypia. HSIL contain CIN2, CIN3 and CIS lesions.

The WHO 2014 classification of glandular lesions, including adenocarcinoma in situ (AIS) and adenocarcinoma (invasive cancer), were updated in 2020 (Stolnicu et al. 2018; Park 2020). The new classification based on the presence of HPV infection includes two groups of adenocarcinomas: HPV-associated endocervical adenocarcinoma (HPVA) and HPV-unassociated endocervical adenocarcinoma (NHPVA) (Table 1).

Table 1.　　Squamous cell tumors and endocervical adenocarcinoma tumor types by WHO 2020.

Squamous cell tumors and precursors	Squamous intraepithelial lesions	LSIL
		HSIL
	Squamous cell carcinoma, not otherwise specified (NOS)	
	Benign squamous cell lesions	

Endocervical adenocarcinoma tumor types	
HPV-associated (HPVA)	Non- HPV-associated (NHPVA)
AIS (usual type)	gAIS
Villoglandular	Atypical LEGH
Mucinous, NOS	Clear cell
Mucinous, intestinal type	Mesonephric
Mucinous, signet ring cell type	Endometrioid
iSMILE	Serous
Micropapillarity	
´Serous´-like	

AIS = adenocarcinoma in-situ, gAIS = gastric type adenocarcinoma in-situ, HPVA= associated endocervical adenocarcinoma, iSMILE=invasive stratified mucin-producing carcinoma, Atypical LEGH = atypical lobular endocervical glandular hyperplasia, LSIL = low grade squamous intraepithelial lesion, NHPVA = HPV-unassociated endocervical adenocarcinoma, HSIL = high grade squamous intraepithelial lesion, NOS= Not Otherwise Specified.

2.2　　Natural history of cervical cancer precursors

The natural history of cervical cancer precursors has been presented as gradually progressing from cellular atypia through CIN to invasive cancer over a period of 10

to 20 years (Holowaty et al. 1999; Burd 2003; Meijer, Snijders, and Brule 2000). CIN is a premalignant condition and refers to squamous neoplasia (World Health Organization 2013).

The natural history of LSIL (CIN1 lesions) is well established. About 90% of CIN1 lesions regress spontaneously due to the transient feature of low-risk human papillomavirus (lrHPV) infections. The rate of progression to cancer is very low. A retrospective study of 1,001 women (mean age 29.2 years) with histological CIN1 showed that at six months follow-up, 49% of lesions had regressed to normal, 45% had persistent CIN1 and only 7% progressed to high-grade lesions. Women with normal pathology at six months were followed up to 12 months. The results demonstrated that 80% of these women remained negative, 16% still had CIN1 and 4% progressed to high-grade lesions (Bansal et al. 2008). In other studies, the reported rates of progression of CIN1 lesions to CIN3 vary from 2% to 10% and regression rates from 44% to 88% (Ostör 1993; Syrjänen et al. 1992; Holowaty et al. 1999) (Table 2).

The natural history of conservatively managed CIN2 lesions was widely described in a systematic review and meta-analysis of 36 studies, including seven randomized trials, 16 prospective cohorts and 13 retrospective cohorts. At the 12-month follow-up, 50% of CIN2 lesions regressed, 32% persisted and 18% progressed to CIN3+. The rate of regression was higher (60%) and the rate of progression lower (11%) among women under 30 years old. Women with an hrHPV-negative test at baseline had a low progression rate (3%–5%), whereas among hrHPV-positive women, the progression rate was 40% at two years. The rate of progression to CIN3 or worse increased with time. At three months, the rate was 5%; at 12 months, 14%; at 24 months, 18%; and at 36 months, 24% (Tainio et al. 2018). An increased risk of CIN2 progression was also demonstrated in earlier studies, ranging from 16% to 35%, with a regression rate of 33%–58% (Nasiell, Nasiell, and Vaclavinková 1983; Chan et al. 2003; Ostör 1993; Holowaty et al. 1999) (Table2).

About 12%–40% of untreated CIN3 progresses to invasive cancer, and 20%–47% regresses spontaneously (Peto et al. 2004; Trimble et al. 2005; McCredie et al. 2008; Munk et al. 2012; Ostör 1993) (Table 2). The risk of invasive cancer increases to 50% at 30 years with persistent disease and decreases to 0.7% at 30 years in treated CIN3 disease (McCredie et al. 2008).

Table 2 demonstrates that the average duration of progression from CIN1 to CIN3 is about 6 years; from CIN2 to CIN 3 it is 2–3 years, and from CIN3 to invasive cancer it is 5–30 years. Correspondingly, the average duration of a

precancerous lesion being observable even if it never progresses is 1–2 years in women with CIN2 lesions and 3–6 months in women with CIN3 lesions.

Adenocarcinoma in situ (AIS) is the recognized premalignant precursor of invasive endocervical adenocarcinoma (AC). The natural history of AIS and AC is not well established. Reliable data on the progression from AIS to AC and the regression rate of AIC is lacking (Polterauer et al. 2013). With respect to age, stage and tumor size, NHPVA endocervical adenocarcinomas occurred in significantly older patients with more advance stage and large size compared to HPVA tumors (Stolnicu et al. 2018).

Table 2. Progression, persistence, and regression rate of CIN1, CIN2 and CIN3 lesions.

Reference	Lesions	Progression rate to CIN3/cancer	Persistence rate	Regression rate
Syrjänen et al. 1992	CIN1	14% at 72 ± 22 months	27% at 72 ±2 2 months	56% at 72 ±2 2 months
Bansal et al. 2008	CIN1	7% at 6 months	25% at 6 months	49% at 6 months
Ostör et al.1993	CIN1	10% to CIN3 1% to invasive cancer	30%	60%
	CIN2	20%	40%	40%
	CIN3	12%		33%
Holowaty et al. 1999	CIN1	2% at 2 years		44% at 2 years
	CIN2	16% at 2 years		33% at 2 years
Nasiell, Nasiell, and Vaclavinková 1983	CIN2	35% at 78 months	15% at 78 months	50% at 78 months
Tainio et al. 2018	CIN2	5% at 3 months 14% at 12 months 18% at 24 months 24% at 36 months	32% at 12 months	50% at 12 months
Munk et al. 2012	CIN2			31% at 113 days
	CIN3			20% at 113 days
	CIN2-3			22% at 113 days
Chan et al. 2003	CIN2			58% at 6 months
	CIN3			47% at 6 months
Trimble et al. 2005	CIN2-3		72% at 15 weeks	28% at 15 weeks
McCredie et al. 2008	CIN3	13.0% at 5 years 20.0% at 10 years 26.1% at 20 years 31.3% at 30 years	16% at 10 years 25% at 20 years	
Peto et al. 2004	CIN3	40% lifetime risk		

CIN1 = cervical intraepithelial lesions grade 1, CIN2 = cervical intraepithelial lesions grade 2, CIN2-3 = cervical intraepithelial lesions grade 2 and grade 3, CIN3 = cervical intraepithelial lesions grade 3

2.3 Epidemiology of cervical cancer and its precursors

2.3.1 Incidence, management, and prognosis of cervical cancer precursors

In developed countries, the annual incidence of CIN1 lesions is 1.2 per 1,000 women and 1.5 per 1,000 for CIN2/3. The highest incidence of CIN1 lesions (5.1 per 1,000) was demonstrated among women aged 20 to 24 years and the highest CIN2/3 incidence (8.1 per 1,000) was observed among women aged 25 to 29 years (Insinga, Glass, and Rush 2004).

Histological LSIL with low malignant potential should be treated conservatively. The *Finnish Current Care Guidelines* recommend observation for women with histological LSIL. Histological LSIL should be treated by loop electrosurgical excision procedure (LEEP) only if lesions persist for more than two years or colposcopy has detected an absent transformation zone. A study of 126 women who underwent LEEP after two years of persistent histological CIN demonstrated that 87% had CIN1 or normal histology, whereas 13% had HSIL histology (CIN2+) (Perkins et al. 2020). Table 3 below shows the CIN3+ risk at one and five years in women with histological CIN1; the risk is increased with previous high-grade cytology or a positive HPV result.

Table 3. CIN3+ risk at one year and five years.

Precolposcopy cytology	Colposcopic biopsy diagnosis	1-year risk, %	5-year risk, %
HPV+/NILMx2	CIN1	0.74	2.8
HPV+/ASC-US	CIN1	0.53	2.6
HPV+/LSIL	CIN1	0.74	2.3
ASC-H	CIN1	1.4	5.6
AGC	CIN1	1.3	3.8
HSIL+	CIN1	3.9	6.6

Modified from Egemen et al. 2020 Risk Estimates Supporting the 2019 ASCCP Risk-Based Management Consensus Guidelines. Journal of Lower Genital Tract Disease 24(2):132-143. ASC-US = atypical squamous cells of undetermined significance, AGS = atypical glandular cell, ASC-H = atypical squamous cells, cannot exclude HSIL, CIN1 = cervical intraepithelial neoplasia grade 1, CIN3+ = cervical intraepithelial neoplasia grade 3 or more severe lesion, HPV+ = human papillomavirus, HSIL+ = high grade squamous intraepithelial lesion or more severe lesion, LSIL = low grade squamous intraepithelial lesion, NILM = negative for intraepithelial lesion or malignancy.

Excisional treatment is recommended for histological HSIL (CIN2 and CIN3) and for AIS. Excisional therapy includes LEEP or large loop excision of the transformation zone (LLEZ), laser conization and cold knife conization. Ablative treatments consist of cryotherapy, laser ablation and thermoablation, but they are not recommended due to compromised evaluability during follow-up. The *Finnish Current Care Guidelines* recommend excision for treatment of HSIL. Hysterectomy is not recommended for primary treatment of HSIL. In women ≤30 years with histological HSIL specified as CIN2, observation with colposcopy every six months up to two years is preferred, but immediate management is also possible. If CIN2 persists for two years, treatment is recommended (Perkins et al. 2020).

After treatment of HSIL, women still have a heightened risk of cervical cancer. A meta-analysis of 97 studies demonstrated that patients treated for CIN2+ had an overall risk of residual disease or recurrence of 6.6%. The relative risk of persistence or recurrence is 4.8 times higher with positive resection margins. However, margin status can predict only 55.8% of residual disease or recurrence, whereas the predictive value of hrHPV testing is 91.0%. With free margins, the risk of CIN2+ after treatment was 0.8% if hrHPV was negative and 3.7% if hrHPV was positive (Arbyn et al. 2017). A positive hrHPV test and abnormal cytology at follow-up after treated HSIL increase the risk of CIN3 at five years after treatment (Table 4). Risk of CIN3+ at five years strongly decreased among HPV negative women after treatment of CIN2 or CIN3 lesions. The risk is 1.7%, 0.91% and 0.44% after one, two or three negative HPV tests, respectively (Egemen et al. 2020).

Table 4.   CIN3+ risk at five years after treatment of histological HSIL (CIN2 and CIN3).

Previous Histology	Current HPV status	Current cytology	CIN3+ risk, %
CIN2 or 3	HPV-negative	NILM	1.7
CIN2 or 3	HPV-negative	ASC-US/LSIL	3.8
CIN2 or 3	HPV-negative	ASC-H/AGC/HSIL+	18
CIN2 or 3	HPV-positive	NILM	12
CIN2 or 3	HPV-positive	ASC-US/LSIL	21
CIN2 or 3	HPV-positive	ASC-H/AGC/HSIL+	63

Modified from Egemen et al. 2020 Risk Estimates Supporting the 2019 ASCCP Risk-Based Management Consensus Guidelines. Journal of Lower Genital Tract Disease 24(2):132-143. ASC-US = atypical squamous cells of undetermined significance, AGS = atypical glandular cell, ASC-H = atypical squamous cells, cannot exclude HSIL, HPV = human papillomavirus, CIN2 = cervical intraepithelial neoplasia grade 2, CIN2 or 3 = cervical intraepithelial neoplasia grade 2 or grade 3, CIN3 = cervical intraepithelial neoplasia grade 3, CIN3+ = cervical intraepithelial neoplasia grade 3 or more severe lesion, HSIL+ = high grade squamous intraepithelial lesion or more severe lesion, LSIL = low grade squamous intraepithelial lesion, NILM = negative for intraepithelial lesion or malignancy.

A Swedish study showed that aging is one of the attributes of high risk of cervical cancer after treatment for CIN3 (Strander, Hällgren, and Sparén 2014). The risk of invasive cancer and mortality among women with previous CIN3 began to rise after the age of 50 years (Table 5).

Table 5.   Standardized incidence ratios and standardized mortality ratios for cervical or vaginal cancer among women with previous CIN3 diagnosis.

Age	Incidence (95%CI)	Mortality (95%CI)
30-39	2.03 (1.83-2.24)	1.52 (1.21-1.89)
40-49	2.62 (2.33-2.94)	2.33 (1.92-2.82)
50-59	5.65 (4.85-6.54)	4.12 (3.18-5.25)
60-69	10.58 (8.54-12.96)	7.60 (5.38-10.43)
Whole cohort	2.39 (2.26-2.53)	2.35 (2.11-2.61)

Modified from Strander, Hällgren, and Sparén 2014 Effect of ageing on cervical or vaginal cancer in Swedish women previously treated for cervical intraepithelial neoplasia grade 3: population based cohort study of long term incidence and mortality. BMI 348,1-11. CI = confidence interval, CIN3 = cervical intraepithelial neoplasia grade 3.

The incidence of AIS is 1.25 per 10,000 women-years (Polterauer et al. 2013). The mean age of patients with AIS is 37 years, whereas that for cervical adenocarcinoma (AC) is 51 years (Baalbergen and Helmerhorst 2014). Excisional treatment of AIS can preserve women's fertility. The *Finnish Current Care Guidelines*

propose excision and later hysterectomy for treatment of AIS. Some institutions prefer cold knife conization or laser conization instead of LLEZ. Excisional management of AIS is a relatively safe option for women who want to preserve fertility. The overall risk of recurrent AIS after conization with negative margins and negative endocervical curettage (ECC) is 5%–10% and risk of invasive carcinoma is 0.1%–5.2% (Östör et al. 2000; Salani, Puri, and Bristow 2009). With positive margins, the recurrence rate increases to 17% (Baalbergen and Helmerhorst 2014). After a radical excision, residual disease was found in 16.5%–20.3% of the secondary specimens (Salani, Puri, and Bristow 2009; Baalbergen and Helmerhorst 2014). In the case of positive margins or positive ECC, reconization should be suggested. After childbearing is concluded, hysterectomy should be recommended due to the high recurrence risk (Polterauer et al. 2013).

2.3.2   Incidence, mortality, and survival of cervical cancer

In 2018, according to an online database of global cancer statistics (GLOBOCAN), cervical cancer was the fourth most common cancer in women worldwide, ranking after breast cancer, colorectal cancer, and lung cancer with an estimated 569,847 new cases worldwide. It was the eighth most common cancer in women in Europe with an estimated 61,072 new cases of cervical cancer in Europe (Bray et al. 2018; Ferlay et al. 2018). Cervical cancer is the second most frequently common type of cancer in women aged 15–44 years in the world (Bruni et al. 2019). Cervical cancer is the most diagnosed cancer in 28 countries at the national level in females. The highest regional incidence rates are seen in Swaziland (Southern Africa), with a 43.1 age-standardized rate (ASR) adjusted to the World Standard Population (W) per 100,000 persons per year. In lower Human Development Index (HDI) settings, cervical cancer is the most common cancer after breast cancer. The lowest incidence rates for cervical cancer were reported in Western Asia, with 4.1 ASR (W) per 100,000 (Bray et al. 2018). There is about a tenfold change in the incidence rates across 20 world regions (Figure 1). In 2016, according to a database of cancer statistics for the Nordic countries (NORDCAN) 26-MAR-2019, the incidence of cervical cancer in Finland was the lowest among the Nordic countries, with 4.3 ASR (W) per 100,000. Each year about 180 new cases of cervix uteri cancer are diagnosed in Finland (estimates for 2018). Cervical cancer is the fourth most common cancer in women aged 15–44 years in Finland (Nordcan 2021).

In 2018, cervical cancer was the fourth most common cause of cancer-related deaths among women with an estimated 311,365 deaths from cervical cancer worldwide. It was the tenth most common cause of cancer-related deaths among women in Europe with an estimated 25,829 deaths from cervical cancer (Bray et al. 2018; Ferlay et al. 2018). In Eastern, Western, Middle and Southern Africa, cervical cancer was the leading cause of cancer death among women in 2018 (Arbyn et al. 2020). The highest mortality rates were in Malawi (Eastern Africa), with 30.0 ASR (W) per 100,000 (Bray et al. 2018). In 42 countries, cervical cancer is the leading cause of cancer death at the national level in females, especially in Sub-Saharan Africa and Southeast Asia, while the lowest mortality rates were in Australia/New Zealand, with 1.7 ASR (W) per 100,000 (Bray et al. 2018). (Figure 1). According to the NORDCAN database on 26-MAR-2019, the mortality rate for cervical cancer in Finland was 1.1 ASR (W) per 100,000. Cervical cancer ranks as the 18th leading cause of female cancer in Finland, while it is the fourth leading cause of cancer deaths in women aged 15–44 years (HPV Information Centre 2019).

Figure 1. Region-specific incidence and mortality age-standardized rates for cancer of the cervix in 2018.
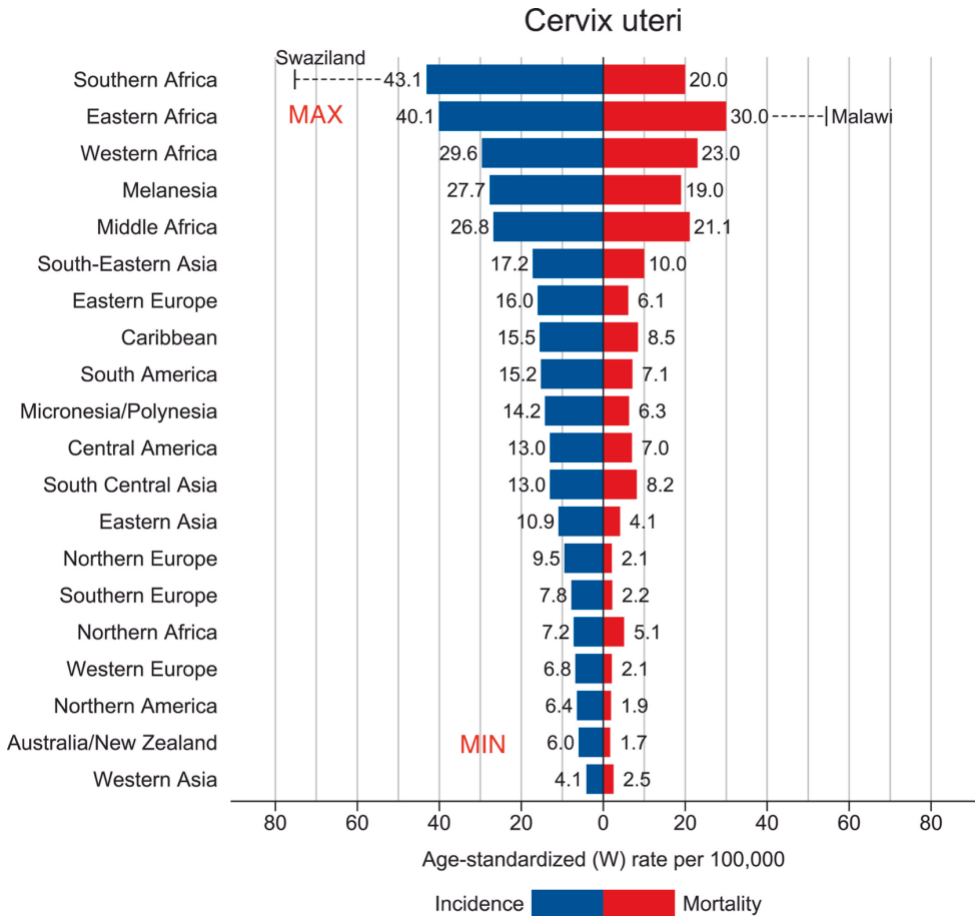
According to the GLOBOCAN, the five-year cervical cancer survivors in 2018 was estimated at 1,474,265 worldwide (39.0 per 100,000 women) and 190,814 survivors in Europe (49.7 per 100,000 women) (Ferlay et al. 2019). In 2011–2015, according to the NORDCAN database, the relative five-year survival of cervical cancer in Finland was 66% (95% CI: 63%–69%), whereas the risk of acquiring the disease before 75 years of age was 0.4% (Nordcan 2021).

2.3.3   Trends

Over the last few decades, incidence and mortality rate of cervical cancer have decreased in all developed and many developing nations due to the organized cervical cancer screening programs.

According to the GLOBOCAN 2018 database, incidence and mortality rates from cervical cancer have diminished in North America, many European countries and Australia. Overall, the incidence of cervical cancer in the United States decreased from 9.6 per 100,000 women in 2000 to 7.4 per 100,000 in 2012 (Yoo et al. 2017). On overage, cervical cancer mortality rates decreased by 1.5% annually between 2000 and 2012 (Yoo et al. 2017). From 2012 through 2016, the incidence of cervical cancer was stable in the United States (Henley et al. 2020).

According to the NORDCAN data from 2018, age-standardized incidence rates of cervical cancer decreased from 19.09 to 8.6 per 100,000 women in Nordic countries from 1960 to 2015 and age-standardized mortality rates decreased from 7.67 to 1.59 per 100,000 women. In Finland, cytological cervical cancer screening was started in 1963 and was extended to the whole country in the early 1970s. The implementation of the screening program has led to a nearly 70% decrease in incidence (14.20 ASR [W] per 100,000 in 1960; 4.27 ASR [W] per 100,000 in 2015) and an over 80% decrease in mortality from cervical cancer (7.16 ASR [W] per 100,000 in 1960; 1.10 ASR [W] per 100,000 in 2015). Data from 1995–2009 on 602,225 women from 51 countries show that cervical cancer survival was stable or slightly increased in most countries between 1995–1999 and 2005–2009. The five-year age-standardized net survival between 1995 and 2009 was stable in Finland, ranging from 66.2% to 65.3%, increased in Sweden from 65.0% to 67.8%, in Norway from 66.7% to 71.4%, in Denmark from 63.1% to 64.8% and in Iceland from 63.6% to 73.1% (Allemani et al. 2015).

Currently, WHO's global strategy is to accelerate elimination of cervical cancer as a public health problem by reducing the global annual age-standardized incidence to four per 100,000 women (World Health Organization 2020; Arbyn et al. 2020). High vaccination rate among girls can help eliminate cervical cancer by the end of the century in the most lower-middle-income countries by the end of the century and by 2055–2059 in high-income countries (Brisson et al. 2020; Arbyn et al. 2020). In lower-middle-income countries, vaccination of 90% of all girls at age nine would reduce the median age-standardized incidence of cervical cancer from 19.8 to 2.1 cases per 100,000 women-years by 2055–2059. Vaccination with screening at 35 and

45 years old would reduce the incidence to 0.7 cases per 100,000 women-years (Brisson et al. 2020). According to WHO, the estimated cervical cancer mortality across 78 lower-middle-income countries was 13.3 per 100,000 women in 2018. The WHO comparative modeling analysis showed that by 2030, vaccination alone has been predicted to decrease mortality of cervical cancer by only 0.1%, but the addition of screening at 35 and 45 years old and cancer management would reduce death from cervical cancer by 34.2% in lower-middle-income countries, compared to the status quo. By 2070, WHO's triple-intervention strategy including vaccination, twice-lifetime screening and cancer management would decrease mortality by 92.3%, avoiding 62.6 million deaths. Over the next century, the WHO strategy would decrease cervical cancer mortality by almost 99%, saving the lives of more than 62 million women (Canfell et al. 2020).

2.4 Etiology of cervical cancer

In 1976/1977, two studies, by Meisels with Fortin and Purola with Savia, reported cervical dysplasia in cells infected by HPV. These investigators were the first to introduce the concept that HPV might be an etiological agent of cervical cancer (Meisels and Fortin 1976; Purola and Savia 1977). In 1982, a group led by Professor Harald zur Hausen proved the causal role of HPV infections in development of cervix cancer (Hausen 2009). Since then, many studies have demonstrated the causal association between persistent high-risk HPV infection and invasive cervical cancer. A study coordinated by the International Agency for Research on Cancer (IARC) showed that HPV DNA was detected in almost all (99.7%) of about 1,000 cases of cervical cancer (Walboomers et al. 1999).

Currently, 201 different HPV types have been officially registered (Poljak et al. 2016). About 40 of these can affect the anogenital tract and upper aerodigestive tract (Villiers et al. 2004; Villiers 2013). Certain types of HPV viruses with high oncogenic potential have been found in cervical cancer, while others are not associated with cancer. Due to different carcinogenic risks, human papilloma virus types are classified into two groups: high-risk (oncogenic) types, which can cause dysplasia or cancer, and low-risk (non-oncogenic) types, which are linked to low-grade dysplasia and genital warts. The most common clinically relevant high-risk papillomavirus types are 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 and the low-risk types are 6, 11, 40, 42, 43, 44, 54, 61, 72 and 81 (IARC 2007).

HPV16 and HPV18 are the two most common aggressive types and have been demonstrated in about 70% of all squamous cervical cancers, in about 50% of cervical intraepithelial neoplasia grade 3 (CIN3) and in 80% of all adenocarcinomas (Smith et al. 2007; Bruni et al. 2017). HPV was found in in 89% of adenocarcinomas in women younger than 40 years and in 43% of adenocarcinomas in women ≥60 years (Burd 2003). Worldwide, HPV16 and HPV18 are estimated to be present in about 41%–67% of HSIL, in 16%–32% of LSIL and in 6%–27% of ASC-US (Clifford et al. 2006). HPV16 causes about 55%–60% of all cases of cervical cancer, whereas HPV18 causes about 10%–15% of cases (Saslow et al. 2012). The other 10 high-risk types of papilloma viruses are associated with an additional 25%–35% of the remaining cervical cancer cases (Saslow et al. 2012). HPV 18 was demonstrated more often in cases of adenocarcinoma than in cases of squamous cell carcinoma (32% vs. 8%) (Sanjose et al. 2010). According to a report from the HPV Information Centre in 2017, the prevalence of HPV16 among squamous cell carcinoma (SCC) and adenocarcinoma was 56.8% (95% CI: 56.4%–57.3%) and 36.1% (95% CI: 34.8%–37.3%), respectively. The prevalence of HPV18 among SCC and adenocarcinoma was 11.6% (95% CI: 11.3%–11.9%) and 34.9% (95% CI: 33.7%–36.1%), respectively (Bruni et al. 2019). In Finland in 2017, according to the HPV Information Centre, the prevalence of HPV16 and HPV18 in SCC was 77.8% (95% CI: 73.2%–81.9%) and 15.6% (95% CI: 12.2%–19.8%), respectively, and in adenocarcinoma was 16.7% (95% CI: 10.8%–24.8%) and 55.6% (95% CI: 46.2%–64.6%), respectively (HPV Information Centre 2019).

2.4.1 Genomic organization and HPV life cycle

Human papillomaviruses (HPVs) are a group of small (55-nm diameter), non-enveloped, epitheliotropic, double-stranded DNA viruses that affect skin or mucosal cells. The human papilloma virus genome codes for eight genes: six nonstructural proteins (E1, E2, E4, E5, E6 and E7) in an early (E) coding region of the genome and two structural capsid proteins (L1 and L2) in a late (L) coding region of the genome (Zheng and Baker 2006). The proteins from the early region can be classified into regulatory genes (E1 and E2), oncogenes (E5, E6 and E7), and E4, which is a biomarker for active virus infection with the replication of viral genome (Wang et al. 2013). E1, E2, E5, E6 and E7 are linked to the early differentiation stage of the epithelium, E4 is represented throughout all stages of differentiation, and L1 and L2

are linked to the final stages of differentiation. E1–E7 are necessary for viral replication and L1 and L2 for virion assembly (IARC 2007). Oncoproteins disrupt normal cell-cycle control. The E6 oncoprotein inhibits p53 with subsequent blockage of apoptosis. The E7 protein inhibits retinoblastoma tumor suppression proteins (pRB) leading to DNA replication (Schiffman et al. 2005; Doorbar 2005).

Figure 2.   Papillomavirus genome organization and its life cycle in cervical epithelium.
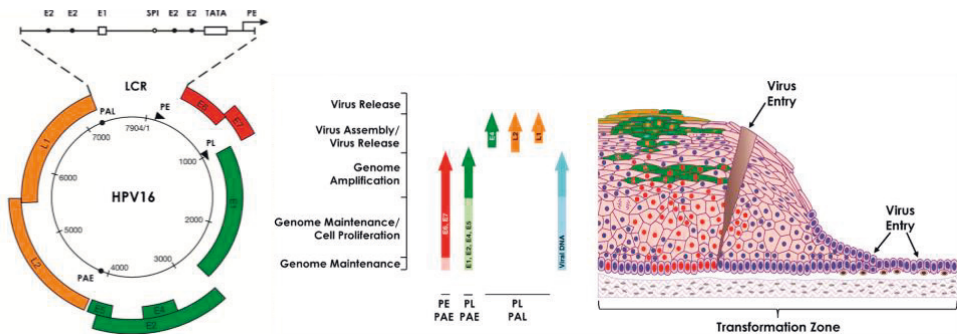
Reprinted from Doorbar et al. 2012 The Biology and Life-Cycle of Human Papillomaviruses. Vaccine 30(5):55-70. Copyright (2020) with permission from Elsevier. HPV16 = human papilloma virus type 16, LCR = long control region,

The HPV life cycle includes the following phases. In the establishment phase, viral particles migrate through the microwounded epithelium to cells in the basal layers, with a low number of copies after amplification of the viral genome. The next phase is the maintenance phase, which is when the viral genome integrates into the host cell chromosomes and induces genome replication. Finally, in the productive phase, abolition of the restrain of cell progression and retarded normal terminal proliferation, genome amplification and packaging, virus synthesis and release take place (McBride 2008; Doorbar 2005; Doorbar et al. 2012).

The development of cervical cancer reflects the HPV life cycle and comprises four steps: high risk HPV types contaminate the cervix transformation zone with metaplastic epithelium, persistence of oncogenic HPV infection, progression of infected epithelium to precancerous lesions and finally progression to cancer with invasive growth through the basement membrane of the epithelium (Schiffman et al. 2007).

2.4.2   Transmission and risk factors

Transmission of HPV infection occurs primarily by skin-to-skin, skin-to-mucosa or mucosa-to-mucosa contact with an infected partner, requiring penetration of HPV to basal cells through tears and abrasions in squamous or mucosal epithelium. At present, HPV infection is the most common sexually transmitted viral infection (Aral, Fenton, and Holmes 2007). It is estimated that more than 80% of sexually active men and women will be infected with HPV during their lifetime (Chrysostomou et al. 2018). Numerous factors may influence the probability of transmission, such as age of first sexual intercourse, number of lifetime sexual partners, concomitant infections, use of condoms, immune suppression, smoking, hormonal contraceptives, viral load and duration of infection (Burchell et al. 2006; Veldhuijzen et al. 2010; Moscicki et al. 2012).

Several natural history studies of HPV infection have reported that about 20%–30% women with HPV infection have multiple types of the virus simultaneously (Méndez et al. 2005; Reiter, Pendergraft, and Brewer 2010). The exact incidence of HPV-infected individuals is difficult to assess because usually virus DNA is present for a short time only and is eliminated without ever developing clinically recognized signs (Baseman and Koutsky 2005; Burchell et al. 2006).

The most important risk factors of developing cervical cancer are the type of HPV virus and long-term persistence of HPV infection. However, a mere presence of oncogenic HPV is not sufficient; the presence of co-factors is required for malignant progression (Bosch et al. 2002; Muñoz et al. 2006; IARC 2007). The established co-factors are tobacco smoking, increased parity, long-term use of oral contraceptive, and HIV co-infection (Bruni et al. 2019). Host immunological and genetic factors together with viral factors contribute to the immune response, but their role in cancer development has not been clearly defined (Sanjosé, Brotons, and Pavón 2018; Brown and Leo 2019; Muñoz et al. 2006).

Early age of sexual debut can be a risk factor for invasive cervical carcinoma (ICC), but the reasons for this association are still unclear (González and Green 2007; Almonte et al. 2008; Plummer, Peto, and Franceschi 2012; Bruni et al. 2019). A pooled analysis of 1864 ICC cases and 1719 controls from eight developing countries showed an increased risk of ICC in women with early age at first sexual intercourse. The risk for ICC is 2.3–2.5-fold in women with sexual debut ≤16 years and the risk is 1.8–2.1-fold for women with sexual debut at 17–20 years old,

compared to women with their first sexual intercourse at ≥21 years (Louie et al. 2009).

HPV is a sexually transmitted infection with a strong link between sexual activity and cervical cancer (Herrero et al. 1990; Berraho et al. 2017; González and Green 2007). A meta-analysis of epidemiological studies recorded a significant increased risk of ICC among women with multiple sexual partners (Odds Ratio [OR] 1.77; 95% CI: 1.50–2.05) (Liu et al. 2015). Previous *Chlamydia trachomatis* infection, indicated by serum antibodies, has also been found to be linked to an increased risk of cervical cancer (Koskela et al. 2000; Anttila et al. 2001; Smith et al. 2004; Lehtinen et al. 2011; Dahlström et al. 2011; Paavonen 2012; Silva, Cerqueira, and Medeiros 2014; Castellsagué et al. 2014; Zhu et al. 2016).

Several studies have demonstrated a direct correlation between high parity and increased risk of cervical cancer (Muñoz et al. 2002; Hinkula et al. 2004; González and Green 2007). According to the IARC-pooled analysis, high parity with seven or more full-term pregnancies in HPV-positive women was associated with a nearly fourfold risk for developing cervical cancer compared to HPV- positive nulliparous women (OR 3.8; 95% CI: 2.7–5.5). This study found no association with the risk of adenocarcinoma or adenosquamous carcinoma (Muñoz et al. 2002). A study in Denmark found no statistically significant association between the number of pregnancies and CIN3+ lesions (hazard ratio (HR) 1.27; 95% CI: 0.82–1.65) among women with hrHPV infection. In contrast, The risk of CIN3+ increased statistically significantly among hrHPV-positive women and women with persistent hrHPV infection who had given birth (HR 1.50; 95% CI: 1.02–1.91 and HR 1.78%; 95% CI: 1.07–2.94 CI) (Jensen et al. 2013).

It has been observed that immunosuppression (after organ transplantation or due to an immunodeficiency disease such as HIV) is an important risk factor which increases the chances of getting cervical cancer (Clifford and Franceschi 2007; Dugué et al. 2013; Sherston et al. 2014; Ghebre et al. 2017).

Asthana *et al.* reported a significant association between HPV infections and the use of oral contraceptives (OCs), with an OR of 1.77 (95% CI: 1.4–2.24) for adenocarcinoma, 1.29 (95% CI: 1.18–1.42) for squamous cell carcinoma and 1.7 (95% CI: 1.18–2.24) for carcinoma in situ (Asthana, Busa, and Labani 2020). Moreno *et al.* demonstrated that up to five years' use of OCs was not correlated with increased risk for cervical cancer (OR 0.73; 95% CI: 0.52–1.03), but the OR increased to 2.82 (95% CI: 1.46–5.42) for women who used OC for 5-9 years and to 4.03 (95% CI: 2.09–8.02) for women who used OC for 10 years or longer (Moreno et al. 2002). Use of an intrauterine device (IUD) decreased the risk of adenocarcinoma compared with

never use (OR 0.41; 95% CI: 0.18–0.93) (Castellsagué et al. 2006). Recent use of a levonorgestrel IUD was related to slightly higher rate of CIN2+ lesions (Relative Risk [RR] 18; 95% CI: 1.05–1.18) but not CIN3+ (RR 1.02; 95% CI: 0.93–1.11) (Averbach et al. 2018).

Tobacco smoking elevated the risk almost 2-fold for SCC but not for adenocarcinoma (Winkelstein 1977; Kjellberg et al. 2000; Plummer et al. 2003; Vaccarella et al. 2008; Gadducci et al. 2011; Roura et al. 2014; Castellsagué et al. 2006). According to the pooled data from eight case-control studies, smoking was not associated with cervical adenocarcinoma (Castellsagué et al. 2006).Relative risks for developing cervical cancer associated with different co-factors are summarized in Table 6.

Table 6. Relative risk of cervical cancer in relation to Chlamydia trachomatis infection, oral contraceptive use, parity, and smoking.

Cofactors	Risk for	
	Squamous cervical cancer, RR	Adenocarcinoma, RR
Age at first intercourse ≤16 years (Louie et al. 2009)	1.8–2.1	
Multiple sexual partners (Liu et al. 2015)	1.77	
Chlamydia trachomatis (Zhu et al. 2016)	2.19–2.21	1.61
(Smith et al. 2004)	1.8	1.0
(Paavonen 2012)	2.0	
Parity (5+) (Berrington De González and Green 2007)	2.08	1.61
(7+) (Muñoz et al. 2002)		
(≥ 2) (Jensen et al. 2013)	3.8	
	1.27	
Oral contraceptives (Asthana, Busa, and Labani 2020) (Asthana, Busa, and Labani 2020)	1.29	1.77
Smoking (Roura et al. 2014)	1.9	

RR = relative risk

2.4.3   Persistence and clearance

About 10%–20% of HPV infections persist latently or progress to a preneoplastic lesion or cancer, and approximately 90% of HPV-infected women clear a specific HPV type after two years of observation (Moscicki et al. 2004; Shanmugasundaram and You 2017; IARC 2007; Sanjosé, Brotons, and Pavón 2018).

Several studies have shown that hrHPV types persist longer than other HPV types. A meta-analysis of 86 studies found that the most persistent types of HPV virus are HPV16, 31, 33 and 53. The median duration of lrHPV persistence is 8.4 months, that of hrHPV in general is 9.3 months, while that of HPV16 and HPV18 are 12.4 and 9.8 months, respectively (Rositch et al. 2013).

The association between age and viral clearance is not completely understood. Some studies have not found association between persistence of HPV infection and age (Muñoz et al. 2004; Mũoz et al. 2009; Trottier et al. 2008; Rositch et al. 2013). Other studies reported that older women had lower rates of persistent HPV infection than younger woman (Bory et al. 2002; Sellors et al. 2003; Rijkaart et al. 2006; Rositch et al. 2013). However, a study with a 67-month follow-up reported the opposite result: the rate of persistent HPV infection was lower among younger women compared with older women (Castle et al. 2005).

2.5   Cytological screening

2.5.1   Cytological classification systems

In 1928, Dr. Georgios Papanicolaou reported cancer cells in cervical smears, and by the early 1960s, Pap smears had become widely used (Vilos 1998). Until the early 2000s, many laboratories continued to use the Papanicolaou classification system for reporting PAP smear results. The system was based on the presence of dysplastic cells. For example, a class I smear was negative for cancer and a class V smear represented cancer (Chatterjee, Gill, and Rac 2000).

In 1989, the National Cancer Institute Workshop on Terminology for Cervical and Vaginal Cytology introduced a new system for reporting Pap smear results named The Bethesda System (TBS) (Soloman 1989). This new terminology should promote communication between the laboratory and the clinician. TBS underlines

that cervical cytology is an incomplete screening tool for adenocarcinoma (Nayar and Wilbur 2017). TBS was revised in 1991, 2001 and 2014.

The most recent TBS describes three major variants: negative for intraepithelial lesions or malignancy (NILM), epithelial cell abnormalities and other malignant neoplasms. Some categories have sub-categories. Epithelial cell abnormalities include abnormal findings in squamous and glandular cells. The last TBS recommended a two-tiered terminology for reporting squamous intraepithelial lesions: low-grade SIL (LSIL) and high-grade SIL (HSIL), which correspond to two alternative pathways an HPV infection can take: either as a transient viral infection in LSIL or as infection with a tendency to progress to precancer and finally to cancer in HSIL (Nayar and Wilbur 2017).

The present TBS system reflects the new two-tiered classification system for histopathological terminology, which also distinguishes two types of lesions: histological LSIL and HSIL (Darragh et al. 2012). This accomplishment should lead to clearer management guidelines.

2.5.2 Management of cytological findings

Most Pap smears are normal; only about 10%–20% are abnormal (ASC-US 6%, LSIL 3%, ASC-H 0.25%, HSIL 0.5%)(Saslow et al. 2002; Nanda et al. 2000). An annual review of the cervical screening program in Finland reported that, in 2018, about 95% of the screened women had a normal screening result, about 4% of the women had borderline test results (ASC-US, LSIL for women under 30 years, or a positive HPV test without referral for further examinations), and 1.2% of the women received a recommendation for further examinations. In 2018, the proportion of women with a serious positive test result (ASC-H+/AGC+) requiring colposcopy was 0.6% in Finland, 1.1% in Sweden, and 1.1% in Norway (Finnish Cancer Registry 2018). According to the Finnish Current Care Guidelines, women with normal screening results are referred for their next screening in five years. The Finnish guidelines recommend follow-up screening for women with borderline cytology (ASC-US, LSIL for women under 30 years, or a positive HPV test in combination with normal or borderline cytology). Colposcopy is recommended for all women with more severe cytological results, such as ASC-H, HSIL, AGC-NOS, AGC-FN or LSIL (for women aged >30 years). The Finnish guidelines recommend colposcopy for women with persistent hrHPV positivity within 24 months (however,

no earlier than 12 months), even if the Pap test result is normal or ASC-US (Käypä hoito 2021).

2.5.3 The use of hrHPV testing in triage of women with ASC-US and LSIL cytology

The optimal clinical management of minor abnormal cytology or ASC-US and LSIL has long been problematic due to the low precancerous potential associated with these cytological abnormalities. According to Egemen *et al,* the immediate risk of CIN3+ for women with ASC-US and LSIL cytology is 0.04% and 1.1%, respectively. The presence of hrHPV infection increases the risk of CIN3+ up to 4.4% and 4.3%, respectively (Egemen et al. 2020).

Three different strategies were investigated for triage of ASC-US and LSIL cytology in the ASCUS-LSIL Triage Study (ALTS) randomized trial: immediate referral to colposcopy at enrollment, triage with hybrid capture 2 (HC2) (referral to colposcopy with either a positive HPV test or HSIL cytology), and triage with repeat cytology (referral to colposcopy with cytological HSIL). Follow-ups were conducted with all patients every six months for two years. The prevalence of hrHPV in women with ASC-US was 48.9% and 83.3% in women with LSIL cytology. The ALTS study demonstrated that the sensitivity of HC2 in the detection of CIN3+ lesions among women with ASC-US cytology was higher compared to repeat cytology: 96.3% (95% CI: 91.6%–98.8%) vs. 85.3% (95% CI: 78.2–90.8), respectively. The HC2 test's superior sensitivity compared to cytology for detecting CIN2+ lesions was demonstrated in women with ASC-US (Table 7). The investigators concluded that hrHPV testing is a viable option for ASC-US triage and that HPV-negative women with ASC-US cytology do not need colposcopy (Solomon, Schiffman, and Tarone 2001; Schiffman and Solomon 2003). According to an interim analysis, hrHPV prevalence among the women with LSIL cytology was too high to allow triage with HC2 in women with LSIL cytology. The ALTS analysis showed that neither HPV testing nor repeat cytology can be suggested as a triage test for LSIL cytology. As a result, hrHPV-positive women with ASC-US cytology and all women with LSIL cytology should be referred for colposcopy (Arbyn et al. 2006; Schiffman and Solomon 2003).

A meta-analysis of HPV triage in women with ASC-US and LSIL outcomes showed the utility of HPV testing for triage of ASC-US cytology but not LSIL cytology. HC2 triage at cutoff ASC-US or worse yielded significantly higher

sensitivity than repeat cytology for detection of CIN2+ lesions with a pooled sensitivity of 1.16 (95% CI: 1.04–1.29) without loss of specificity (Table 7) (Arbyn et al. 2004). A summary of meta-analyses of clinical applications of HPV test concluded that in women with ASC-US cytology, hrHPV triage is more accurate (significantly higher sensitivity and not lower specificity) than triage with repeat cytology in predicting the presence of CIN2+.–In women with LSIL cytology, hrHPV tests does not demonstrate a higher sensitivity for presence of precancerous lesions but does show a significantly lower specificity compared to a repeat cytology (Table 7) (Arbyn et al. 2006). A pilot study from England on triage with hrHPV testing among women with LSIL cytology resulted in a reduction of 74% in the rate of repeat cytology, but referral to colposcopy was more than double (Moss et al. 2006).

A review of the 39 studies evaluated the accuracy of the HC2 assay for predicting CIN2+ and CIN3+ in women with a cytological result as ASC-US and LSIL. The relative sensitivity of HC2 testing to predict CIN2+ and CIN3+ was superior in comparison with repeat cytology triage in ASC-US women (relative sensitivity 1.27; 95% CI: 1.16–1.39 for CIN2+ and 1.14; 95% CI: 1.06–1.22 for CIN3+). The pooled specificity of HC2 and repeat cytology for detection CIN2+ did not differ significantly from each other (relative specificity 0.99; 95% CI: 0.97–1.03). The sensitivity and specificity of HC2 and repeat cytology in identifying CIN2+ lesions in women with ASC-US cytology are presented in Table 7. The investigators concluded that the HC2 assay demonstrated better performance in triage of women with a cytological result as ASC-US (higher sensitivity, similar specificity) compared to repeat cytology. In addition, other hrHPV tests, such as Abbott RealTime PCR, Papillocheck, Cervista and Aptima, were shown to be acceptable for use as a triage for ASC-US cytology (Arbyn et al. 2012).

In women with LSIL cytology, the pooled sensitivity of HC2 for predicting CIN2+ was significantly higher than that of repeat cytology (ratio of 1.23; 95% CI: 1.06–1.4). However, the specificity of HC2 testing in women with LSIL cytology for precancerous lesions was substantially and significantly lower (ratio of 0.66; 95% CI: 0.58–0.75) compared with repeat cytology. Based on those outcomes, HC2 was not recommended as a triage test for women with cytological result as LSIL following European or American guidelines (Table 7) (Wright et al. 2007; Jordan et al. 2008; Arbyn et al. 2012).

Table 7. The sensitivity and specificity of HC2 hrHPV tests and repeat cytology for detection of histological CIN2+ in women with ASC-US and LSIL cytology.

Reference	hrHPV sensitivity for CIN2+ or relative accuracy to repeat cytology %, (95% CI)	Repeat cytology sensitivity for CIN2+, %, (95% CI)	hrHPV specificity for CIN2+ or relative accuracy to repeat cytology, %, (95% CI)	Repeat cytology specificity (or relative) for CIN2+, %, (95% CI)
	ASC-US cytology			
ALTS (Solomon, Schiffman, and Tarone 2001)	95.9%	85.0%	N.A.	N.A.
Arbyn 2004 (Arbyn et al. 2004)	94.8% (92.7%–96.9%) 1.16 (1.04–1.29)	81.8% (73.5%–84.3%)	67.3% (58.2%–76.4%)	57.6% (49.5%–65.7%)
Arbyn 2006 (Arbyn et al. 2006)	92.5% (90.1%–94.9%) or 1.14 (1.08–1.20)	N.A.	62.5% (57.8%–67.3%) or 0.99 (0.88–1.10)	N.A.
	LSIL cytology			
Arbyn 2004 (Arbyn et al. 2004)	N.A. or 1.69 (1.54–1.85)	45.7% (34.0%–7.4%)	N.A. or 0.71 (0.64–0.80)	89.1% (82.1%–96.2%)
Arbyn 2006 (Arbyn et al. 2006)	97.2% (95.6%–98.9%) or 1.07 (0.92–1.25)	N.A.	28.6% (22.2%–35.0%) or 0.60 (0.36–0.99)	N.A.
Arbyn 20012 (Arbyn et al. 2012)	95.4% (94.0%–96.5%) or 1.23 (1.06–1.4)		27.8% (23.8%–32.1%) or 0.66 (0.58–0.75)	

ALTS = the ASCUS-LSIL Triage Study, ASC-US = atypical squamous cells of undetermined significance, CI = confidence interval, CIN2+ = cervical intraepithelial neoplasia grade 2 or more severe lesion, hrHPV = high-risk human papillomavirus, LSIL = low grade squamous intraepithelial lesion, N.A. = not available.

The Finnish Current Care Guidelines recommend an hrHPV test for the triage of ASC-US in women aged 30–60 years. If the hrHPV test is positive, colposcopy is recommended. If the hrHPV test is negative, colposcopy is not required, and the woman can be referred for her next screening in five years. (Käypä hoito 2021).

2.5.4   The use of HPV tests in management of women after treatment of CIN

Women after treatment for HSIL have an approximately 10% (95% CI: 6.7%–13.8%) treatment failure rate—that is, residual or recurrent disease of high-grade lesions (Arbyn et al. 2006). A long-term multi-cohort study from the Netherlands demonstrated that the five-year and 10-year cumulative risk of CIN2+ lesions was 16.5% (95% CI: 13.0%–20.7%) and 18.3% (95% CI: 13.8%–24.0%) in women after management of HSIL (Kocken et al. 2011).

To predict treatment outcomes, hrHPV testing is used in many countries in follow-up after treatment for high-grade lesions, as hrHPV testing has higher sensitivity with not less specificity compared with cytology alone. The sensitivity of HPV testing and cytology to predict development of CIN2+ lesions after treatment varies, ranging between 92% and 100% and between 72% and 79%, respectively. The specificity of HPV testing and cytology to predict post-treatment CIN2+ was 80%–95% and 84%–95%, respectively (Kocken et al. 2012; Ryu et al. 2012; Arbyn et al. 2012).

Several studies have demonstrated that a positive HPV test in follow-up after treatment is linked to an increased risk of residual disease and disease recurrence. The five-year risk of recurrent CIN2+ disease is 1.0%–3.7% in HPV-negative and 2.7%–4.2% in women with negative cytology (Katki et al. 2013; Kocken et al. 2011). The 10-year risk of CIN3+ in follow-up after treatment of HSIL was 2.1% in hrHPV-negative women and 2.8% in women with a negative Pap result (Arbyn et al. 2012).

According to the Finnish Current Care Guidelines, hrHPV-negative women with NILM, ASC-US or LSIL cytology at 24 months after treatment of high-grade lesions can return to routine cervical cancer screening (Käypä hoito 2021).

2.6   HPV test for primary cervical screening

The incidence and mortality from cervical cancer has declined by around 50% since the mid-1970s in more developed countries in general and around 80% in Finland after the implementation of cervical cancer screening programs (Nieminen, Kallio, and Hakama 1995; Hristova and Hakama 1997; Anttila et al. 1999; Arbyn et al. 2009; Lönnberg et al. 2012). Cytological screening may have reached its limits, as reports

from several countries with Pap screening programs show that the incidence and mortality from cervical cancer have stabilized or begun to rise again (Arbyn et al. 2009; Lönnberg et al. 2012; Kok et al. 2012; Arbyn et al. 2020). The reason for this trend might be weaknesses in cytology, namely the dependence on the quality of the sample and subjectivity in the interpretation of the results, which limits the sensitivity of cytological screening. Moreover, the Pap screening is less sensitive for detection and prevention of cervical adenocarcinomas (Sasieni et al. 2009; Adegoke, Kulasingam, and Virnig 2012; Tota et al. 2017). A pooled analysis of European and Canadian studies reports the sensitivity of conventional cytology for detecting of high-grade precancerous lesions to be between 51% and 53%, while specificity ranges from 96% to 98%. This means that up to 50% of women with precancerous lesions may have normal cytology (Cuzick et al. 2006; Tota et al. 2017).

Since the mid-1990s, HPV testing has been started to be used as a test for cervical cancer screening (Franco 2003). The sensitivity of primary HPV screening for detection of high-grade lesions is higher by 23%–43% in comparison with cytology, but the specificity is 5%–8% lower (Bulkmans et al. 2007). A large overview of European and North American studies demonstrated that the sensitivity of the HPV test for CIN2+ in primary cervical cancer screening was higher in comparison with cytological screening (96.1% versus 53.0%). The overall specificity of HPV tests for CIN2+ lesions was 90.7%, with a range from 76.5% to 95.5%, versus 96.3% for cytology (Cuzick et al. 2008). Studies with targeted European, African, Asian, Latin American and North American populations have shown that testing for HPV has 27% greater sensitivity than cytology in detecting high-grade cervical lesions but 8.4% less specificity (Franco 2003).

A meta-analysis of randomized and nonrandomized trials showed higher sensitivity (relative sensitivity 1.29; 95% CI: 1.18–1.39) and lower specificity (relative specificity 0.94; 95% CI: 0.92–0.96) of the HPV test as a screening test for detection of CIN2+ in comparison with cytological screening (Richardson, Tota, and Franco 2011). A meta-analysis of studies from North American and European on primary HPV screening demonstrated that the sensitivity of primary HPV tests for excluding CIN2+ was higher by average 23%–43% in comparison with cytological screening (Arbyn et al. 2012).

Current technologies offer three categories of HPV testing based on detection of a viral DNA fragment, mRNA or protein. DNA-based genotyping tests identify by amplification HPV DNA (Meijer et al. 2009; Bhatla and Singhal 2020). An mRNA-based tests detect expression of HPV oncoproteins E6 and E7, a marker of disease progression. A protein-based test recognizes the protein p16INK4a which are over-

expressed in the majority of cervical cancers and precancerous lesions (Richardson, Tota, and Franco 2011). There are approximately 250 different commercial HPV tests available (Bhatla and Singhal 2020). Every candidate HPV test chosen for clinical use undergoes a comparison with a clinically validated reference (Meijer et al. 2009). A clinically validated reference HPV test is represented in the overview of the European and North American studies on HPV testing in primary cervical cancer screening (Cuzick et al. 2008). International guidelines for clinical validation of a candidate HPV test with Meijer's and VALCENT (Validation of HPV Genotyping Test) criteria are designed to validate HPV assays applicable for cervical screening (Bonde et al. 2018; Meijer et al. 2009). The HPV tests accepted for cervical screening in Finland are presented in Table 8.

Table 8.   Overview of the accepted HPV tests in Finland.

DNA based HPV assays	RNA based HPV assays
Hybrid Capture - (HC2) (Qiagen)	APTIMA assay (Hologic)
Cervista HPV-HR (Hologic)	
Cervista (Hologic)	
Onclarity HPV assay (BD)	
HPV-Risk assay (Self-Screen BV)	
Cobas 4800 (Roche)	
Xpert HPV (Cepheid AB)	
RealTime high-risk HPV assay (Abbot)	
Anyplex II HPV HR (Seegene Inc)	
PapilloCheck (Greiner Bio-One)	

Modified from Finnish Cancer Registry, www. https://cancerregistry.fi/.

2.6.1   Primary HPV screening versus cytology

Four randomized controlled trials (RCTs) have analyzed a comparison of primary hrHPV screening versus conventional cytology: the FINNISH trial in Finland (Leinonen et al. 2012), the Compass trial in Australia (Canfell et al. 2017), the HPV for Cervical Cancer Screening (HPV FOCAL) trial in Canada (Ogilvie et al. 2017), and the New Technologies for Cervical Cancer (NTCC) Phase II trial in Italy (Ronco et al. 2008; 2010) (Table 9).

Table 9.   Overview of the FINNISH, COMPASS, FOCAL and NTCC Phase II trials.

Trials	Arms	CIN2+, %	CIN3+, %	Colposcopy rate
FINNISH (Leinonen et al. 2012)	HPV	HPV vs. CC 1.71 (1.43–2.03) for CIN2	HPV vs. CC 1.62 (1.28–2.06) for CIN3	1.2
	CC			1.1
COMPASS (Canfell et al. 2017)	I HPV*	1.0	0.7	3.8
	II HPV**	1.2	0.8	3.9
	LBC	0.1	0.1	2.7
FOCAL (Ogilvie et al. 2017)	HPV	1.7	0.8	5.9
	LBC	1.0	0.5	3.1
NTCC Phase II (Ronco et al. 2008; 2010)	HPV	0.56	0.24	7.8
	CC	0.22	0.11	2.8

CC = conventional cytology, CIN2+ = cervical intraepithelial neoplasia grade 2 or more severe lesion, CIN3+ = cervical intraepithelial neoplasia grade 3 or more severe lesion, I HPV* = human papillomavirus screening with liquid-based cytology triage, II HPV** = human papillomavirus screening with dual-stained cytology triage, LBC = liquid-based cytology, NTCC = the New Technologies for Cervical Cancer trial.

All the RCTs demonstrated a higher detection rate of precancerous lesions (CIN2+ and CIN3+) in hrHPV-screened women in comparison with women screened by cytology in the first screening round. According to the Finnish study, more CIN2 (RR 1.72; 95% CI: 1.43–2.03) and CIN3 or AIS lesions (RR 1.62; 95% CI: 1.28–2.06) were detected in the HPV screening arm compared with the conventional screening arm. However, the rate of invasive cervical cancer was nonsignificantly higher in the hrHPV-screened arm (RR 1.87; 95% CI: 0.83–4.20). In this study, colposcopy rates were almost equal in both screening arms (Leinonen et al. 2012). The Compass trial in Australia showed a 10-fold increase in the detection rate of CIN2+ lesions in women screened with hrHPV test compared to those who were screened with liquid-based cytology (LBC). The overall colposcopy referral rate was higher in both HPV screening groups compared to the LBC-screened group (Canfell et al. 2017).

In the HPV FOCAL study for all age cohorts, the detection rates of CIN2+ and CIN3+ lesions at the baseline screen were equal in the HPV and LBC arms, but at the end of round one (baseline and 12-month follow-up) the rates of CIN2+ and

CIN3+ detection were significantly higher in the HPV arm compared to the liquid-based cytology arm (CIN2+: 16.5/1,000 vs. 10.1/1,000 and CIN3+: 7.5/1,000 vs. 4.6/1, respectively). The colposcopy referral rate after the initial screening for all age cohorts was significantly higher in women screened with HPV testing compared with women screened with LBC (58.9/1,000 vs. 30.9/1,000 (Ogilvie et al. 2017).

The outcomes from the NTCC Phase II study showed that the relative sensitivity of HPV testing for CIN2+ versus conventional cytology was higher among women aged 25–34 years than among women aged 35–60 years, at 3.50 (95% CI: 2.11–5.82) and 1.92 (95% CI: 1.28–2.87), respectively. Direct referral of all the HPV-positive women to colposcopy in the NTCC Phase II trial resulted in a high colposcopy rate in the HPV-screened arm (Table 9).

2.6.2 Primary HPV screening versus HPV testing with cytology

A summary of a meta-analysis demonstrated that primary HPV screening identified 23% more CIN2, CIN3, or cancer lesions compared to cytology at cut-off ASC-US. Adding cytology to the HC2 screening increased the sensitivity for CIN2+ and CIN3+ by 7% (95% CI: 6%–8%) and 4% (95% CI: 3%–5%), respectively, and reduced the specificity by 5% (95% CI: 4–6%) and 7% (95% CI: 5–9%), respectively, compared to HC2 screening alone (Arbyn et al. 2006).

Six years later, Arbyn et al. published their next meta-analysis with almost similar results. This meta-analysis of 49 studies, including eight randomized trials, showed that adding HC2 to cytology increased the sensitivity for detection of CIN2+ and CIN3+ by on average 42% and 33%, respectively, compared with cytology alone at cutoff ASC-US, and decreased the specificity by 6% and 8%, respectively. Adding cytology to HC2 increased the sensitivity for CIN2+ and CIN3+ by 5% (95% CI: 4%-7%) and 2% (95% CI: 1%-2%) and decreased the specificity by 5% (95% CI: 4%-6%) and 7% (95% CI: 5%-8%), respectively, compared to HPV testing alone (Arbyn et al. 2012).

The benefits of cytology co-testing were observed in a population-based study with 331,818 women aged 30 years and older. The five-year cumulative incidence of cervical cancer was 7.5 per 100,000 per year among women with a normal Pap smear result, 3.8 per 100,000 women per year among HPV-negative women, and 3.2 per 100,000 among women who had HPV-negative and Pap negative results. Risk of CIN3+ lesions was smaller among HPV-negative women compared to only Pap-negative women over three years (0.063% vs. 0.17%; $p = 0.001$) and five years (0.17%

vs. 0.36%; $p = 0.02$). Over three years, the cumulative risk of CIN3+ lesions among HPV-positive women with abnormal cytology was increased by 6.9% (10% vs. 3.1%) and over five years by 6.2% (12.1% vs. 5.9%; $p < 0.0001$). However, normal cytology did not decrease the incidence of CIN3+ among HPV-negative women, at either three year (0.047% vs. 0.063%; $p = 0.6$) or five years (0.16% vs. 0.17%; $p = 0.8$). Investigators concluded that the HPV test identified the cumulative risk of CIN3+ more distinctly compared to the cytological test, and there was only a partial benefit of cytology triage (Katki et al. 2011).

Data from 1,208,710 women aged 30 years and older demonstrated that adding cytology to HPV-negative results increased the detection rate of precancer by only 3.5% and cancer by 5.9% (Schiffman et al. 2018).

The American Society of Colposcopy and Cervical Pathology has recommended additional cytology triage for all HPV-positive women, regardless of genotype, and this can help guide recommendations for management. If a woman has an immediate CIN3+ risk of 4% or greater, immediate colposcopy or treatment is indicated (Perkins et al. 2020; Egemen et al. 2020). Egemen *et al.* evaluated the immediate risk of CIN3+ in a prospective longitudinal cohort of more than 1.5 million women who participated in HPV screening (Egemen et al. 2020). Some information from this study is represented in Table 10.

Table 10.   Immediate risk of CIN3+ for abnormal screening results.

Previous history	Current HPV result	Current cytology result	CIN 3+ immediate risk, %
Unknown	HPV+	NILM	2.1
Unknown	HPV+	ASC-US	4.4
Unknown	HPV+	LSIL	4.3
HPV-	HPV+	ASC-US	2.0
HPV-	HPV+	LSIL	2.1
HPV+, NILM	HPV+	NILM	4.1
HPV+, ASC-US	HPV+	ASC-US	5.4
HPV+, LSIL	HPV+	LSIL	5.0

Modified from Egemen et al. 2020 Risk Estimates Supporting the 2019 ASCCP Risk-Based Management Consensus Guidelines. Journal of Lower Genital Tract Disease 24(2):132-143. ASC-US = atypical squamous cells of undetermined significance, CIN = cervical intraepithelial neoplasia, HPV = positive for human papillomavirus, LSIL = low grade squamous intraepithelial lesion, NILM = negative for intraepithelial lesion or malignancy.

2.6.3 Cytology with hrHPV testing versus cytology alone

Four RCTs have analyzed the comparison of cytological screening and cytological screening with the addition of hrHPV test: the Randomised Trial in Screening to Improve Cytology (ARTISTIC) in the United Kingdom (Kitchener et al. 2009), the Population-Based Screening Study (POBASCAM) in the Netherlands (Rijkaart et al. 2012), SWEDESCREEN in Sweden (Naucler et al. 2007), and the NTCC Phase I trial in Italy (Ronco et al. 2006; 2010) (Table 11).

Table 11. Overview of the POBASCAM, ARTISTIC, SWEDESCREEN and NTCC phase I trials.

Trials		CIN2+, %	CIN3+, %	Colposcopy rate
POBASCAM (Rijkaart et al. 2012)	CC arm	1.1	0.7	2.7
	HPV arm	1.3	0.9	3.0
ARTISTIC (Kitchener et al. 2009)	LBC arm	2.17	1.31	20.4
	HPV arm	2.46	1.27	79.6
SWEDESCREEN (Naucler et al. 2007)	CC arm	1.2	0.8	1.7
	HPV arm	1.8	1.2	1.8
NTCC Phase I (Ronco et al. 2006; 2010)	CC arm	0.41	0.23	1.46
	HPV arm	0.81	0.32	10.0

CC = conventional cytology, CIN = cervical intraepithelial neoplasia, HPV = Human papillomavirus, LBC = liquid-based cytology, NTCC = the New Technologies for Cervical Cancer trial.

HC2 was used in the ARTISTIC and NTCC studies, while GP5+/GP6+ PCR was used in the POBASCAM and SWEDESCREEN. Conventional cytology was used in all studies except ARTISTIC, which used liquid-based cytology. The data from these studies are shown in Table 11. In the POBASCAM study, no significant differences in the detection rate of CIN2+ and CIN3+ lesions were registered between the intervention and control groups (Rijkaart et al. 2012). The same result was found in the ARTISTIC study. Additionally, the ARTISTIC study showed a considerably increased rate of colposcopy in the revealed group (HPV) compared with the concealed group (LBC) (79.6% vs. 20.4%). In the first screening round, 51% more cases of CIN2+ and 31% more cases of CIN3+ were registered in the intervention (HPV) group than in the control (cytology) group in the SWEDESCREEN study (Naucler et al. 2007). The NTCC Phase I study has also shown a higher detection rate of precancerous lesions in the HPV testing group in

comparison to the cytology group (Ronco et al. 2006; 2010). During the first screening round, the colposcopy rate was significantly higher in the HPV arm in the NTCC Phase 1 and ARTISTIC studies (Kitchener et al. 2009; Ronco et al. 2006; 2010).

2.6.4   The level of evidence of HPV-based screening

Outcomes for the second screening rounds from the four RCTs (SWEDESCREEN, POBASCAM, ARTISTIC, NTCC) investigating HPV primary screening demonstrated a consistent decrease in the incidence of CIN3+ lesions at the second screening rounds among women with primary HPV screening in comparison with cytological screening with a pooled detection ratio rate of 0.43 (95% CI: 0.33–0.56) (Table 12) (Arbyn et al. 2012). In the Italian study, in women older than 35 years, no cases of invasive cervical cancer were found in the second round among women with an HPV primary test, whereas nine cases were detected among women with primary cytology screening (Ronco et al. 2010). In the second screening rounds, HPV primary screening detected persistent CIN2 and CIN3 lesions earlier and reduced the incidence of CIN2 and CIN3 compared with cytological screening (Kitchener et al. 2009; 2011; Naucler et al. 2009; Ronco et al. 2010; Rijkaart et al. 2012; Arbyn et al. 2012).

The data from the randomized controlled studies further show that HPV-based primary screening offers an additional 60%–70% greater protection against the development of invasive cervical carcinoma in comparison with cytological screening. A pooled analysis of the trials demonstrated that the rate of invasive cancer was similar, 0.79 (95% CI: 0.46–1.36) in both HPV and cytological screening arms only during the first 2.5 years, and after that the rate of invasive cancer significantly decreased to 0.45 (95% CI: 0.25–0.81) in the HPV screening arm. Moreover, the cumulative incidence of invasive cervical carcinoma was significantly lower at 3.5 and 5.5 years after screening in women who had a HPV negative entry test compared to women who had a cytology negative entry test: 4.6 per 10^5 and 8.7 per 10^5 at 3.5 and 5.5 years, respectively, in the HPV-screened arm, and 15.4 per 10^5 and 36.0 per 10^5, respectively, in the cytology-screened arm. These outcomes show that a five-year interval for HPV-based screening is more accurate compared to a three-year interval for cytology (Ronco et al. 2014) (Figure 3). Additionally, HPV

testing reduced the incidence of cervical adenocarcinomas even more effectively than it reduced squamous cell carcinoma (rate ratio: 0.31 vs. 0.78, respectively) (Ronco et al. 2014).

The results of the seven prospective HPV studies from Germany, Sweden, Denmark, the UK, France and Spain demonstrated that after six years, the cumulative incidence of CIN3+ lesions was significantly lower among women who had a baseline HPV-negative than among women who had a baseline Pap-negative (0.25% vs. 0.95%). The rates at a three-year screening were 0.12% versus 0.51%, and at five years were 0.19% versus 0.83%. Additionally, after six years the cumulative incidence of CIN3+ was 34% (95% CI: 26.8%–45.4%) among women with a positive HPV test and abnormal cytology; 10% (95% CI: 6.2%–15.1%) among women with a positive HPV test and normal cytology; and 2.7% (95% CI: 0.6%–6.0%) among women with a negative HPV test and abnormal cytology (Dillner et al. 2008).

According to a study from rural India, the rate of incidence and mortality from advanced cervical cancer was significantly less in the HPV screened group compared with the unscreened control group after a single round of HPV testing. The Indian trial did not have subsequent screening rounds, and the eight-year passive follow-up time showed that the incidence of invasive cervical cancer was significantly lower in women with primary HPV negative result than in women with primary negative cytology result or a negative result of visual inspection of the cervix with acetic acid (VIA). The eight invasive cervical cancers among 24,380 HPV-negative women were detected after eight years from screening, 22 among 23,762 women who had normal cytology and 25 among 23,032 women who had a negative result of VIA, with age-standardized rates of 3.7, 15.5 and 16.0 cases of invasive cervical cancer per 100,000 person-years, respectively. The incidence of advanced cervical cancer (stage II+) and of mortality from cervical cancer was significantly lower in the HC2-screened arm compared with the control arm, in which no screening was offered with a hazard ratio (HR) of 0.47 (95% CI: 0.32–0.69) and 0.52 (95% CI: 0.33–0.83), respectively. The incidence and mortality from advanced cervical cancer did not significantly decline in the cytology-screened group or in the VIA group compared with the unscreened control group. The results of the Indian trial demonstrated HPV testing provides a great protection against advanced invasive cancers and significantly declines the mortality from cervical cancer compared with the unscreened population (Sankaranarayanan et al. 2009).

A review of 40 studies with more than 140,000 women demonstrated that cervical cancer screening identifies 20 women with precancerous lesions per every 1,000

screened women. The HPV screening test will properly recognize 18 of these 20 women and will miss two. The Pap test will recognize 15 of these 20 women and will miss five. Cervical cancer could occur in women with missed test screening. Clearly, the HPV test detects precancerous changes more accurately (Koliopoulos et al. 2017).

Table 12. Meta-analysis of the main outcomes from randomized trials comparing HPV-based and cytological primary cervical cancer screening in the second screening round among women who were HPV-negative versus cytology-negative at enrollment.

Study	Detection ratio rate of CIN3+ (95% CI)	Detection ratio rate of cervical cancer (95% CI)
(Naucler et al. 2007)	0.53 (0.29–0.98)	0.14 (0.01–2.77)
(Kitchener et al. 2009)	0.52 (0.28–0.97)	
(Ronco et al. 2010)	0.34 (0.15–0.75)	0.05 (0.00–0.29)
(Rijkaart et al. 2012)	0.39 (0.27–0.56)	0.17 (0.04–0.74)
Overall	0.43 (0.33–0.56)	0.13 (0.04–0.44)

Modified from Arbyn et al. 2012 Evidence Regarding Human Papillomavirus Testing in Secondary Prevention of Cervical Cancer. Vaccine 30(5):88-99. CIN = cervical intraepithelial neoplasia.

Figure 3. Cumulative detection of invasive cervical carcinoma.
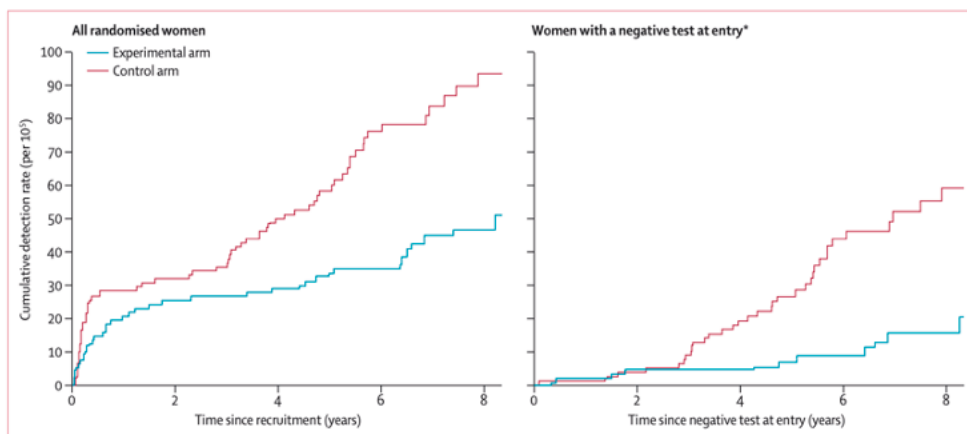
Reprinted from Ronco et al. 2014 Efficacy of HPV-Based Screening for Prevention of Invasive Cervical Cancer: Follow-up of Four European Randomised Controlled Trials. The Lancet 383(9916):524–32. Copyright (2020), with permission from Elsevier

2.6.5 Harms of screening

Potential harms of primary HPV screening include significantly increased rate of false positive results, increased referrals to colposcopy, increased rate of performed biopsies, and more detection of non-progressive CIN2+ lesions (Arbyn et al. 2010). Primary HPV screening detected a significantly higher number of HPV-positive tests compared with abnormal cells in cytology screening. The majority of HPV-positive results (>90%) are eliminated by the immune system without consequences in six to 22 months and do not cause cervical cancer (Chrysostomou et al. 2018). The HPV test will properly recognize 881 women from every 1,000 screened women (99 women will have overdiagnosis). The Pap test will properly recognize 885 women from every 1,000 screened women who have a lesion (95 women will have overdiagnosis). False positives can increase referrals for colposcopy and biopsy and can lead to unnecessary treatment of healthy women (Koliopoulos et al. 2017).

According to data from the ARTISTIC, NTCC and Compass studies, the colposcopy rates were significantly higher in the HPV screening arms in the first screening round compared with cytology screening (Kitchener et al. 2009; Ronco et al. 2006; 2008; 2010; Canfell et al. 2017). In comparison to cytology primary screening, overdiagnosis of regressive CIN2 lesions is considerably higher in women younger than 35 years within the HPV primary screening setting (Arbyn et al. 2010).

3   AIMS OF THE STUDY

This study was undertaken to assess the real-life use of the hrHPV test as a tool in triage of low-grade or repeat equivocal cytological abnormalities, and especially as the primary screening test in an organized cervical cancer screening program. The specific aims were as follows:

1. To evaluate the real-life performance of HPV testing in the triage of low-grade squamous intraepithelial lesions (LSIL) and repeat atypical squamous cells of undetermined significance (ASC-US) (I).

2. To evaluate the initial performance of HPV testing with cytology triage in the setting of an organized routine screening program in comparison with Pap screening (II).

3. To further evaluate the outcomes of primary HPV screening for cervical cancer prevention in the framework of an organized cervical cancer screening program, three years of experience (III).

4. To assess outcomes in the second five-yearly screening round of the HPV-based screening program (IV).

4 MATERIALS/PATIENTS/SUBJECTS AND METHODS

4.1 Materials/Patients/Subjects and study design

For study I, the study data was retrospectively collected from patients who presented with repeat ASC-US and LSIL cytology at the Department of Obstetrics and Gynecology at Tampere University Hospital from 2004 to 2012. Three pregnant women were excluded from the study. A total of 127 women with repeat ASC-US lesions and 118 women with LSIL were tested for hrHPV. All women underwent a colposcopic examination. We aimed to evaluate the sensitivity and the specificity of hrHPV testing for detection of CIN2+ lesions among women with repeat ASC-US and LSIL cytology.

For studies II, III and IV, eligible women invited for cervical screening were traced from the Population Information System by their year of birth. Studies II, III and IV included women living in the city of Tampere; study III also comprised women from seven municipalities surrounding the city of Tampere. Women at 30 years of age screened with the Pap test were excluded from the studies on the basis of age. Numerical data of the study participants are given in Tables 13, 15, 17 and 19.

In study II, we evaluated the outcomes in the detection rate of precancerous lesions and rate of colposcopies in the first round of primary HPV screening in 2012 in comparison with the last Pap screening in 2011 in Tampere. In 2012, a total of 8,076 women received an invitation for primary HPV screening, with an attendance rate of 70%. HPV and Pap tests were administered for all participating women. The pap smear result was analyzed only among women who tested positive for HPV. All women with HPV positive result and LSIL+ cytology were referred to colposcopy. HPV-positive women with normal or borderline cytology (ASC-US, AGC-NOS) were advised to repeat both the HPV and Pap tests in 12 months.

In the re-screening year, all HPV-positive women (regardless of cytology) were referred to colposcopy and all HPV-negative women with normal cytology were scheduled back for the next screening round. Colposcopy was advised for all HPV-

negative negative women with LSIL+ cytology or repeat borderline cytology (ASC-US, AGC-NOS).

In 2011, of the 8,219 women invited, 5,814 women participated in the last cytological-based cervical cancer screening. All women with LSIL+ cytology were referred to colposcopy. All participants with borderline cytology (ASC-US, AGC-NOS) were referred for a repeat Pap test in 12 months. In the re-screening year, colposcopy was recommended for all women with LSIL+ or repeat borderline cytology.

In study III, we analyzed the specificity and relative sensitivity of the HPV test with cytological triage compared to primary cytology screening based on the outcomes from the primary HPV screening in the city of Tampere and the Pap screening in Tampere surroundings from 1 January 2012 to 31 December 2014. Overall, 17,770 women out of 24,692 (72%) were participated in a primary HPV test, and 15,605 women out of 22,016 (71%) in the Pap screening. In both screening groups, all women with abnormal test results were investigated in the same way according to the schedule outlined above.

In study IV, we compared the rate of colposcopies and the rate of HSIL lesions among women who participated in both HPV screening rounds. To analyze the results from the second screening round, we investigated the same group of women over a period of seven years, which enabled us to follow he effectiveness of the new screening method. We followed the 35- to 55-year-old age cohort of women who attended in the first HPV screening round and the same cohort in the second screening round when women were five years older (i.e., 40–60 years old). We excluded women at age 60 due to the absence of screening results for women at age 65. Altogether, 4,571 women 35–55 years old attended the first HPV screening round, and in 2017, 4,807 women 40–60 years old attended the second HPV screening round. All participants with negative HPV tests were referred back to routine HPV screening in five years. At the screening year, colposcopy was recommended for all HPV+/LSIL+ women. HPV-positive women with borderline or normal cytology were recalled for repeat hrHPV and Pap test after 12–16 months. In the re-screening year, all HPV-positive women, regardless of Pap test results, were referred to colposcopy. We investigated the rate of performed colposcopies and the rate of histological HSIL+ lesions in all the age groups.

4.2   Methods

In all studies, cytology was performed using conventional Pap smears. The cytological results were reported according to the 2001 Bethesda System in studies I and II (Solomon et al. 2002) and according to the New Bethesda 2014 System in studies III and IV (Nayar and Wilbur 2015).

The Hybrid Capture II (HC2) High-Risk DNA test (Qiagen, Gaithersburg, MD, USA), identifying 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) was used in study I, before 1 October 2010. Since 1 October 2010, the Abbott RealTime (ART) hrHPV assay (Abbott Molecular, Des Plaines, IL, USA) with concurrent distinction of HPV-16 and HPV-18 from 12 other HPV types for 14 high-risk HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) was offered for primary HPV screening.

In studies II, III and IV, the HPV DNA test used was the Abbott RealTime test. In studies I, II and III, the cervical histopathology was reported using the CIN nomenclature (R. M. Richart 1973). In study IV, the cervical histopathology was described using the fourth edition of the WHO Classification of Tumors of Female Reproductive Organs (2014), which recommended a two-tiered nomenclature for non-invasive HPV-associated squamous proliferation: LSIL and HSIL (Kurman et al. 2014).

4.3   Statistical analysis

In study I, Confidence Intervals Analysis (CIA)–Program Version 2.1.2 (University of Southampton, Southampton, UK) was used for calculation of sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) with 95% confidence intervals. Other statistical analyses were carried out using IBM SPSS STATISTICS for Windows Version 20.0 (released 2011) (IBM Corp., Armonk, NY, USA)

In study II, relative risks (RR) of CIN2+ and CIN3+ lesions with 95% confidence intervals (CIs) between the screening groups were calculated using CIA version 2.2.0. (http://www.som.soton.ac.uk/cia/). Other statistical analyses were performed using IBM SPSS Statistics version 23 (IBM Cop., Armonk, NY, USA).

In study III, the relative sensitivity was calculated from the number of CIN2+ and CIN3+ lesions divided by the number of participants in the screening. Specificity was calculated by divining the number of women with a negative

screening test by the number of screened women who did not have CIN2+ or CIN3+ lesions. The positive predictive value (PPV) was calculated as the proportion of women with CIN2+ and CIN3+ lesions among women referred to colposcopy. The relative sensitivity, specificity, PPV and relative risks (RRs) were also calculated using CIA version 2.2.0 (http://www.som.soton.ac.uk/cia/). Attendance rates were compared with the Cochran-Armitage trend test using StatXact version 4.0.1 (Cytel Software Comparation, ISBN 1-889592-05-6, Cytel, Inc., Cambridge, MA, USA).

In studies I, II and III, Pearson's chi-square and Fisher's tests were used when comparing the outcome groups and the proportion of detected lesions.

In study IV, RRs and differences between percentages with 95% CIs between the screening groups were carried out using R Statistical Software Package (Version 3.6.2, R Core Team [2019]. R: A language and environment for statistical computing, Vienna, Austria. https://www.R-project.org/).

In all studies, any p-value < 0.05 was considered statistically significant.

4.4 Ethical considerations

All studies were carried out in accordance with the conditions of the Declaration of Helsinki and were approved by the Regional Ethics Committee of the Expert Responsibility Area of Tampere University Hospital (25 June 2013, identification number ETL R 13094). No informed consent of individual women was required for the studies, as they were conducted as part of routine clinical work (I) or within the routine screening program (II, III and IV).

5   SUMMARY OF THE RESULTS

5.1   The sensitivity and specificity of hrHPV testing for detection of CIN2+ lesions in triage of women with repeat ASC-US, and LSIL

In study I, colposcopy resulted in 8.7% CIN2+ histology among women with repeat ASC-US cytology and 14.4% CIN2+ histology among women with LSIL cytology (Table 13). High-risk papilloma virus (hrHPV) was present more often in women with LSIL cytology in comparison with ASC-US cytology (68.6% vs. 40.9%) (Table 13). In both groups, hrHPV was detected more frequently among the youngest participants (aged 29 years or younger), 77.3% in the ASC-US group and 81.2% in the LSIL group (Table 13). In each group among women with CIN2+ histology, only one false-negative hrHPV result was identified, while all women with CIN3+ histology had a hrHPV-positive test result in both groups.

Table 13. Distribution of cytology, hrHPV and CIN2+ results in the study population.

Age, years	Cytology		HPV+		CIN2+	
		n	n	%	n	%
<30	ASC-US	22	17	77.3	4	18.2
	LSIL	32	26	81.2	8	25.0
≥30	ASC-US	105	35	33.3	7	6.7
	LSIL	86	55	64.0	9	10.5
Total	ASC-US	127	52	40.9	11	8.7
	LSIL	118	81	68.6	17	14.4

Reprinted from Veijalainen et al. 2015 High risk HPV testing in the triage of repeat ASC-US and LSIL. Acta Obstetricia et Gynecologica Scandinavica 94(9):931-936. Copyright (2020), with permission from Elsevier. ASC-US = atypical squamous cells of undetermined significance, CIN2+ = cervical intraepithelial neoplasia grade 2 or more severe lesion, HPV= human papillomavirus, LSIL = low grade squamous intraepithelial lesion.

The sensitivity and specificity of Abbott RealTime (ART) for histological CIN2+ lesions among women with repeat ASC-US and LSIL cytology are presented in Table 14.

Table 14. The sensitivity and specificity of hrHPV testing in the triage of repeat/persistence ASC-US and LSIL for detection of CIN2+ lesions.

Triage group	Sensitivity, % (95% CI)	Specificity, % (95% CI)
Repeat ASC-US	90.9% (58.7%–98.5%)	63.8% (54.4%–72.5%)
LSIL	94.1% (71.2%–99.0%)	35.6% (85.9%–99.6%)

Reprinted from Veijalainen et al. 2015 High risk HPV testing in the triage of repeat ASC-US and LSIL. Acta Obstetricia et Gynecologica Scandinavica 94(9):931-936. Copyright (2020), with permission from Elsevier. ASC-US = atypical squamous cells of undetermined significance, CI =confidential interval, HPV = positive for human papillomavirus, LSIL = low grade squamous intraepithelial lesion.

5.2 The results of the first hrHPV screening round in comparison with cytological screening

In study II, the attendance rate in the HPV and Pap screenings of all age groups is presented in Table 15. In both groups, the attendance rate ranged from 61% to 75%.

The attendance rate was the lowest among the 35-year-old participants. The total attendance rate remained at the same level in both screened groups, 70%.

Table 15. Age distribution and attendance rate in the Pap and HPV-screened groups.

Age, years	Pap group			HPV group		
	Invited, n	Attended		Invited	Attended	
	n	n	%	n	n	%
35	1.432	853	60	1.409	862	61
40	1.142	774	68	1.092	684	63
45	1.367	953	70	1.398	960	69
50	1.400	1.049	75	1.379	1.034	75
55	1.389	1.027	74	1.385	1.031	74
60	1.489	1.158	78	1.413	1.066	75
Total	8.219	5.814	71	8.076	5.637	70

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In the index year of each screening round, the referral rate to colposcopy was 1% of the participants in both screened groups. About 6% of participants in the HPV-screened group and 13% in the Pap-screened group were invited to follow up at 12 months. At this reflex screening, 160 (3%) additional colposcopies were performed in the HPV-screened group and 57 (1%) in the Pap group, with total colposcopy rates of 4% and 2%, respectively.

Overall, 6.0/1,000 CIN2+ lesions were detected during the first round of the HPV screening and 2.9/1,000 CIN2+ lesions in the Pap screening. HPV screening resulted in the detection of twice as many CIN2 or worse lesions compared with conventional cytology (RR 2.06; 95% CI: 1.15–3.69), whereas the increase of CIN3+ lesions was not statistically significant (RR 1.75; 95% CI: 0.80–3.82). The histological outcomes from both screenings are presented in Table 16 below.

Table 16. The total number of CIN2+ and CIN3+ lesions in the Pap and HPV screenings.

Histology	Pap group (n = 5,814) Detection rate per 1,000 women		HPV group (n = 5,637) Detection rate per 1,000 women		RR HPV vs. Pap (95% CI)
CIN2+ Original screening	11	1.9	16	2.8	1.50 (0.70–3.23)
CIN2+ Reflex screening	6	1.0	18	3.2	3.09 (1.23–7.77)
Total	17	2.9	34	6.0	2.06 (1.15–3.69)
CIN3+ Original screening	5	0.9	10	1.8	2.06 (0.70–6.03)
CIN3+ Reflex screening	5	0.9	7	1.2	1.44 (0.46–4.54)
Total	10	1.7	17	3.0	1.75 (0.80–3.82)

Reprinted from Veijalainen et al. 2016 Human papillomavirus test with cytology triage in organized screening for cervical cancer. Acta Obstetricia et Gynecologica Scandinavica 95(11):1220-1227. Copyright (2020), with permission from Elsevier. CIN2+ = cervical intraepithelial neoplasia grade 2 or more severe lesion, CIN3+ = cervical intraepithelial neoplasia grade 3 or more severe lesion, HPV = human papillomavirus.

In both screening groups, most precancerous CIN2+ lesions were identified in women 45 years of age or younger, 24 cases in the HPV-screened group and 11 cases in the Pap-screened group.

5.3 The first three years of HPV-based screening in the city of Tampere

The data from the first screening round in the city of Tampere showed that the primary HPV-based cervical screening provided better clinical sensitivity for CIN2+ with a nonsignificant increase in detection of CIN3+ compared to conventional cytological screening. The aim of study III was to verify the outcomes of HPV screening in a larger screening population.

The attendance rate was 72% in the primary HPV screening and 71% in the cytological screening groups, with attendances ranging from 61% to 78% (Table 17).

The lowest attendance rate was registered among the youngest participants in both screening groups. Overall, 7.1% of the 1,265 women with positive HPV results underwent a conventional cervical smear triage with results of 1.1% (190 out of 17,770 women) LSIL+ cytology and 6.0% (1,075 out of 17,770 women) borderline or normal cytology. In the Pap-screened group, LSIL+ cytology was detected in 1% of the attending women and borderline cytology in 6.4%. A reflex screening at 12 months was recommended for 6.0% of women in the HPV-screened group and for 6.4% in the Pap-screened group (RR 0.94; 95% CI: 0.87–1.03).

Table 17. Age distribution and attendance rate in the Pap and the HPV groups.

Age, years	Pap group			HPV group		
	Invited	Attended		Invited	Attended	
	n	n	%	n	n	%
35	3.893	2.383	61	4.497	2.847	63
40	3.749	2.519	67	3.456	2.318	67
45	3.787	2.638	70	4.007	2.836	71
50	3.718	2,793	75	4.224	3.236	77
55	3.373	2.540	75	4.222	3.219	76
60	3.496	2.732	78	4.286	3.314	77
Total	22.016	15.605	71	24.692	17.770	72

Reprinted from Veijalainen et al. 2018 Implementation of HPV-based cervical cancer screening in organized regional screening programme: 3 years of experience. Cytopathology 30(2), 150-156, Copyright (2021), with permission from Elsevier. HPV-human papillomavirus.

The incidence of CIN2+ lesions in the HPV- versus Pap-screened groups was 0.75% (134 of 17,770) versus 0.31% (48 of 22,016), respectively, and CIN3+ lesions was 0.47% (83 of 17,770) versus 0.17% (27 from 15,605), respectively (Table 17 and Table 18).

Table 18. The incidence rate of CIN2+ and CIN3+ lesions in the Pap and the HPV groups.

Age, years	Pap group, n		HPV group, n	
	CIN2+	CIN3+	CIN2+	CIN3+
35	15	10	41	28
40	15	9	26	16
45	8	3	22	12
50	5	4	20	15
55	3	0	12	6
60	2	1	13	6
Total	48	27	134	83

The relative sensitivity of primary HPV screening with a conventional cervical smear triage compared with conventional cytological screening was 2.45 (95% CI: 1.76–3.41) for CIN2+ and 2.70 (95% CI: 1.75–4.16) for CIN3+. The specificity of the HPV screening alone in comparison with the Pap screening for CIN2+ was 93.6% (95% CI: 0.93%–0.94%) versus 99.2% (95% CI: 0.98%–0.99%), respectively, and 93.3% (95% CI: 0.93%–0.94%) versus 99.1% (95% CI: 0.98%–0.99%) for CIN3+, respectively.

The rate of performed colposcopies was 4.4% in the HPV-screened group and 2.1% in the Pap-screened group (RR 2.07; 95% CI: 1.83–2.35).

5.4 Comparison of the first and second HPV screening rounds

In the first screening round, 70% of the 8,076 invited women attended the screening, and 71% out of 8,331 attended the second screening round. The lowest attendance rate was observed among the 35-year-old participants: 61% in 2012 and 64% in 2017. Of all screening participants, 69% were 35–55 years old in 2012, while in 2017 the participation rate was 73% when this cohort became 40–60 years of age (Table 19).

Table 19.   Attendance rate in the first and the second screening rounds.

Age, years	The first screening round			The second screening round		
	Invited	Attended		Invited	Attended	
	n	n	%	n	n	%
35	1,409	862	61	1,702	1,095	64
40	1,092	684	63	1,327	944	71
45	1,398	960	69	1,112	766	69
50	1,379	1,034	75	1,394	1,000	72
55	1,385	1,031	74	1,391	1,049	75
60	1,413	1,066	75	1,405	1,048	75
Total	8,076	5,637	70	8,331	5,902	71

The percentage of women with a positive HPV test result was 7% in 2012 and 6.7% in 2017 ($p = 0.51$). In the index year of both screening rounds, about 1% of the attending women were hrHPV-positive and had a positive cytology triage, resulting in a colposcopy rate of 1.1% in 2012 and 0.7% in 2017 ($p = 0.70$). Performed colposcopies resulted in the detection of 19 (0.3%) histological HSIL+ lesions in 2012 and 11 (0.2%) HSIL+ lesions in 2017.

Six percent of the screened women with a positive hrHPV test result had a negative cytology triage in the first screening round, while the proportion was 5.9% in the second screening round. These women were referred to re-screening after an average of 12–16 months, with a compliance rate of 84% in the first screening round and 81% in the second screening round.

At the re-screening, the clearance rate of HPV infection was 38% (1.9% of the women who attended) in the first screening round and 52% (2.5% of the women who attended) in the second screening round ($p = 0.004$). Of the participants in the first and second screening rounds, 3.1% and 2.3%, respectively, had a persistent hrHPV infection, and they were referred to colposcopy with a compliance rate of 96% and 94%, respectively. As a result, in the re-screening year, the rate of performed colposcopies was 3.0% in the first screening round and 2.1% in the second screening round ($p = 0.01$).

At the reflex screening, 0.4% additional cases of histological HSIL+ were detected in each screening round (19 cases in the first screening round and 18 cases in the second screening round).

In the first screening round, the total rate of histological high-grade lesions among women 35–55 years old was 0.8%, decreasing to 0.6% five years later when they were 40–60 years old (Table 20).

Table 20. Rate and relative risk (RR) of histological HSIL+ in the first (I) and second (II) screening rounds in women who were 35–55 years old in 2012 and 40–60 years old in 2017.

Age, years, I round	HSIL, I round, n (%)	Age, years, II round	HSIL, II round, n (%)	RR (95% CI) II vs. I round	p-value
35	16 (1.9)	40	10 (1.1)	0.57 (0.26–1.25)	0.170
40	5 (0.7)	45	4 (0.5)	0.71 (0.19–2.65)	0.743
45	7 (0.7)	50	5 (0.5)	0.69 (0.22–2.15)	0.573
50	6 (0.6)	55	3 (0.3)	0.49 (0.12–1.97)	0.339
55	4 (0.4)	60	7 (0.7)	1.72 (0.50–5.86)	0.548
Total	38 (0.8)	Total	29 (0.6)	0.73 (0.45–1.17)	0.220

In cohort of women screened twice with the HPV test, the total colposcopy rate significantly declined in the second screening round, from 4.0% to 2.9%. The age-specific analysis demonstrated that the decline was greatest in the youngest age cohort, or 35 years old women in the first screening round and 40 years old in the second screening (Table 21).

Table 21. Rate and relative risk (RR) of performed colposcopies in the first (I) and second (II) screening rounds in women who were 35–55 years old in the first screening round and 40–60 years old in the second screening round.

Age, years, I round	Colposcopy, I round, n (%)	Age, years, II round	Colposcopy, II round, n (%)	RR (95% CI) II vs. I round	p-value
35	43 (5.0)	40	22 (2.3)	0.47 (0.28–0.77)	0.003
40	33 (4.8)	45	21 (2.7)	0.57 (0.33–0.97)	0.038
45	32 (3.3)	50	30 (3.0)	0.90 (0.55–1.47)	0.700
50	39 (3.8)	55	34 (3.2)	0.86 (0.55–1.35)	0.552
55	38 (3.7)	60	32 (3.1)	0.83 (0.52–1.32)	0.466
Total	185 (4.0)	Total	139 (2.9)	0.71 (0.57–0.89)	0.002

6 DISCUSSION

6.1 ASC-US and LSIL triage (Study I)

Among women with ASC-US and LSIL cytology, the risk of precancerous lesions is low, as 85%–91% of the lesions regress without treatment after 36–54 months (Schlecht et al. 2001; 2003; Moscicki et al. 2004). As discussed in the review of the literature, the risk of CIN2+ among women who had ASC-US as their cytology result is 6%–10% and 12%–16% with LSIL cytology (Arbyn et al. 2004; Solomon, Schiffman, and Tarone 2001; Schiffman and Solomon 2003). According to the literature, HPV status predicts the risk of precancerous lesions. The five-year risk of CIN2+ for hrHPV-positive ASC-US (19%) was almost at the same level as that for LSIL (20%). In contrast, the five-year risk of CIN2+ for hrHPV-negative ASC-US (1.4%) was notably smaller than for LSIL (5.3%) (Demarco et al. 2017).

The proportion of ASC-US and LSIL cytology among screened women is quite high. According to the annual Finnish review of cervical cancer in 2018, borderline test results were detected in 4% of women who attended the cervical screening program, with the range between hospital districts between 1% and 12% (Finnish Cancer Registry 2018). It is extremely important to find the best way to triage women with minor cytology abnormalities.

In Finland, hrHPV testing is recommended as a triage test for women with repeat ASC-US, due to significantly superior sensitivity and similar specificity in comparison with triage by conventional cytology. Data from other studies showed that the sensitivity to identify histological CIN2+ at the cutoff ASC-US with different hrHPV assays varied from 86.2% to 96.2%, and the specificity varied from 33.3% to 67.3% (Table 22). The outcomes from study I follow the findings from the presented studies. We conclude that the real-life experience demonstrated that the hrHPV test is a useful tool for triage in women with ASC-US cytology.

Table 22. Sensitivity and specificity of hrHPV test in triage of repeat ASC-US cytology to detect CIN2+ lesions.

Studies	HPV test	Sensitivity %, (95% CI)	Specificity %, (95% CI)
Arbyn et al., (Arbyn et al. 2004)	HC2	94.8 (92.7–96.9)	67.3 (58.2–76.4)
Cuzick et al., (Cuzick et al. 2006)	HC2	93.1 (91.1–95.19	62.3 (57.6–67.1)
Huang et al., (Huang et al. 2009)	ART	96.2 (86.8–99.5)	33.3 (25.6–41.8)
Arbyn et al., (Arbyn et al. 2013)	HC2	90.9 (85.7–94.4)	60.7 (52.9–68.0)
Study I (Veijalainen et al. 2015)	ART and HC2	90.9 (58.7–98.5)	63.8 (54.4–72.5)
White et al., (White et al. 2016)	HC2	94.7 (93.4–96.0)	64.4 (60.8–68.1)
Macedo et al., (Macedo et al. 2019)	mRNA (Aptima and PreTect HPV-Proofer)	86.2 (84.0–88.3)	58.3 (56.8–59.3)

ART = Abbott RealTime PCR assay, CI = confidence interval, HC2 = Hybrid Capture 2, HPV = human papillomavirus, LSIL = low-grade squamous intraepithelial lesion, mRNA = messenger ribonucleic acid.

The sensitivity of the hrHPV test for identifying CIN2+ lesions among women with LSIL as their cytological result is high, ranging from 83.7% to 98.3%, but the specificity is low, ranging from 22.1% to 30.6% (Table 23). Outcomes from study I are in line with the previous findings, with a sensitivity of 94.1% (95% CI: 71.2%–99.0%) and a specificity of 33.6% (95% CI: 24.9%–43.3%).

Table 23. Sensitivity and specificity of hrHPV test in triage of LSIL cytology to detect CIN2+ lesions

Studies	HPV test	Sensitivity %, (95% CI)	Specificity %, (95% CI)
Cuzick et al., (Cuzick et al. 2006)	HC2	97.2 (95.6–98.8)	30.6 (22.7–38.6)
Huang et al., (Huang et al. 2009)	ART	98.3 (90.6–100.0)	22.1 (14.9–30.9)
Arbyn et al., (Arbyn et al. 2013)	HC2	96.2 (91.4–98.3)	27.7 (20.9–35.7)
Study I (Veijalainen et al. 2015)	ART and HC2	94.1 (71.2–99.0)	35.6 (26.4–45.8)
White et al., (White et al. 2016)	HC2 for repeat LSIL	91.4 (89.6–93.1)	35.3 (32.0–38.6)
Macedo et al., (Macedo et al. 2019)	mRNA (Aptima and PreTect HPV-Proofer)	83.7 (81.0–86.1)	51.5 (49.5–53.5)

ART = Abbott RealTime PCR assay, CI = confidence interval, HC2 = Hybrid Capture 2, HPV = human papillomavirus, LSIL = low-grade squamous intraepithelial lesion, mRNA = messenger ribonucleic acid.

In the triage of the women with LSIL cytology, the hrHPV test presented a significantly higher sensitivity in detecting precancerous lesions but poor specificity in comparison with the triage of the women with ASC-US cytology. Therefore, the use of HPV triage in women with LSIL cytology is not advised due to the high prevalence of hrHPV infections in women with LSIL cytology (Cuzick et al. 2006; Huang et al. 2009; Arbyn et al. 2013; Veijalainen et al. 2015; White et al. 2016; Macedo et al. 2019).

6.2 HPV test in organized screening for cervical cancer (Studies II-IV)

6.2.1 Sensitivity of HPV testing

In study II, the initial outcomes from the first HPV screening round in Tampere are in concordance with the results from cross-sectional and several randomized HPV

trials. The first round of HPV screening in our study detected twice as many CIN2+ lesions (RR 2.06; 95% CI: 1.15–3.68) in comparison with cytological screening. The NTCC study had similar findings in women 35–60 years old. In round one of the NTCC phase II study, the detection rate of CIN2+ was 2.13 (95% CI: 1.50–3.03) times higher in women who were screened with the hrHPV test than in women who were screened with cytology (Ronco et al. 2010). Furthermore, at the baseline screening, the detection rate of CIN2+ lesions also significantly increased in the HPV-screened women by 51% in the SWEDESCREEN trial and by 25% in the POBASCAM trial (Naucler et al. 2007; Rijkaart et al. 2012). In the Finnish study, 54% more CIN2 lesions were detected in women aged 25–65 years who were screened with the hrHPV test compared to the women who were screened with the Pap test (Leinonen et al. 2012). The relative sensitivity of the HC2 test compared with cytology to detect CIN2+, pooled from 18 cross-sectional studies, was 1.23 (95% CI: 1.13–1.23) (Arbyn et al. 2006). The updated meta-analysis of data from the six randomized trials presented consecutively and significantly superior sensitivity of HPV testing for detection of CIN2+ lesions in comparison with conventional cytological test in industrialized countries, with a detection rate ratio of 1.39 (95% CI: 1.23–1.57), with the exception of the ARTISTIC study (Arbyn et al. 2012). The relative sensitivity of the HPV testing for CIN2+ versus LBC in the ARTISTIC trial was only 1.06 (95% CI: 0.87–1.28) (Kitchener et al. 2009; Arbyn et al. 2012). Possible reasons for low sensitivity in the ARTISTIC study could be the overdiagnosis of lesions detected by LBC and incomplete compliance with follow-up in HPV-positive women (Arbyn et al. 2012).

The findings of several randomized trials also showed higher sensitivity of HPV testing for identifying of CIN3+ lesions in comparison with conventional cytology at the baseline screening. However, some studies could not demonstrate the superiority of HPV testing in the detection of CIN3+ (Table 11). The relative detection rate (HPV vs. cytology) for CIN3+ was significantly higher in the NTCC trial and the POBASCAM trial, with an RR of 2.08 (95% CI: 1.47–2.95) and 1.70 (95% CI: 1.15–2.51), respectively (Bulkmans et al. 2007; Ronco et al. 2010). The Finnish randomized trial showed that the HPV test did not increase detection of CIN3 or cancer in comparison with cytology. At the level of CIN3 and invasive cancer, the RR was 1.10 (95% CI: 0.57–2.12) and 0.99 (95% CI: 0.20–4.89), respectively (Kotaniemi-Talonen et al. 2008). Later, another prospective randomized Finnish trial with a larger screening cohort showed a slight increase of CIN3 or AIS precancerous lesions in the HPV-screened arm, with an RR of 1.32 (95% CI: 1.09–1.59). However, for cervical cancer, the RR was 0.81 (95% CI: 0.48–1.37) (Leinonen

et al. 2012). A nonsignificant increase of CIN3+ lesions was demonstrated in the SWEDESCREEN trial (RR 1.31; 95% CI: 0.92–1.85) (Naucler et al. 2007). The CIN3+ detection rate was not increased in the ARTISTIC trial (RR 0.97; 95% CI: 0.75–1.25) (Kitchener et al. 2009). The pooled relative sensitivity (HPV vs. cytology) for CIN3+ from five randomized trials was 1.28 (95% CI: 1.09–1.51) (Arbyn et al. 2012). The results from study II also failed to show a significantly higher detection rate of CIN3+ lesions in the first HPV screening round compared to the last Pap screening round (RR 1.75; 95% CI: 0.80–3.82) (Veijalainen et al. 2016). However, in the large study III, the HPV test also had a significantly higher sensitivity for detection of CIN3+ in comparison with Pap test (RR 2.06; 95% CI: 1.17–3.41) (Veijalainen et al. 2019).

In conclusion, the present data and data from the literature strongly imply that HPV-based screening is superior to cytological screening in detecting both CIN2+ and CIN3+ lesions. In addition, more recent randomized and nonrandomized trials lend support to our outcomes and demonstrate that compared to cytological screening, HPV testing is less likely to miss high-grade cervical lesions (Richardson, Tota, and Franco 2011; Arbyn et al. 2012; Koliopoulos et al. 2017).

6.2.2   Implications for colposcopy referrals

Given the poorer specificity of HPV testing, the number of positive test results requiring further management was significantly higher. Data from many studies implicate a higher colposcopy rate associated with baseline primary HPV screening versus cytological screening. A meta-analysis of eight randomized controlled trials, five cohort studies and one individual participant data study demonstrated increased colposcopy rates in the initial screening round, ranging from 1.2% to 7.9% for HPV screening and 1.1% to 3.1% for cytological screening (Melnikow et al. 2018). The colposcopy rate results in study II (3.8% in the HPV-screened group and 2.0% in the Pap-screened group) and study III (4.4% and 2.1%, respectively) are in line with the meta-analysis mentioned above (Veijalainen et al. 2016; 2019).

Our data from baseline screening still demonstrated the high colposcopy rate in the HPV-screened arm. We suggested that referring all HPV-positive women at re-screening to colposcopy could explain the 2.3-fold increased colposcopy rate in the HPV-screened group.

6.2.3 Specificity and positive predictive value of HPV testing

In study III, we calculated the ART specificity and positive predictive value (PPV) in detecting CIN2+ and CIN3+ lesions. Its specificity for CIN2+ was 93.6% (95% CI: 93.2%–93.9%). This is in concordance with outcomes from other studies that demonstrated that the specificity of ART for CIN2+ histological lesions varied from 90.3% (95% CI: 89.4%–91.1%) to 93.3% (95% CI: 92.4%–94.2%) (Poljak and Ostrbenk 2013). The specificity of ART alone for CIN3+ (93.3%) achieved the level of specificity required for well-validated HPV testing, which was proposed to be at least 85% for CIN3+ (Stoler et al. 2007). According to a Finnish randomized study, the specificity of HC2 with cytology triage for detecting CIN2+ and CIN3+ lesions was 99.1% (95% CI: 99.0%–99.2%) and 98.8% (95% CI: 98.7%–99.0%), respectively (Kotaniemi-Talonen et al. 2008). In our study, the specificity of ART for CIN2+ (99.2; 95% CI: 98.8–99.6)) and CIN3+ (99.1; 95% CI: 98.6–99.5) lesions achieved the same level after adding cytology triage (Veijalainen et al. 2019).

The PPV of the ART test with cytology triage for CIN2+ was 33.9% (95% CI: 25.3%–43.5%) and 21.9% (95% CI: 16.4%–28.4%) for CIN3+. The PPV of ART for CIN2+ has varied from 15.6% (95% CI: 11.3%–20.7%) to 20.6% (95% CI: 13.2%–29.7%) across studies (Poljak et al. 2011; Chung, Hahm, and Lee 2014). In the study of Iftner *et al.*, the PPV for CIN2+ increased to 84.2% for the separate detection of HPV16 with the ART test (Iftner et al. 2016). The Finnish randomized study demonstrated a higher PPV of HC2 with cytology triage for CIN2+ and CIN3+, at 51% (95% CI: 44.6%–57.4%) and 32.4% (95% CI: 26.6%–38.6%), respectively (Kotaniemi-Talonen et al. 2008).

The high sensitivity and acceptable PPV of ART with cytological triage for the detection of high-grade cervical intraepithelial lesions demonstrates its suitability as a screening test for cervical cancer.

6.2.4 Comparison with other regional real-life studies

Other studies of the implementation of primary HPV screening in routine practice have been published. The VASCAR study from Canada showed a near threefold increase in histological HSIL detected in women who were screened with the hrHPV test in comparison with women who were screened with conventional cytology (RR 2.78; 95% CI: 2.1–3.7) for CIN2+) and a 33% increase in the rate of colposcopy referrals (Louvanto et al. 2014). In the VASCAR study, in contrast to the present

study, the attendance of participants in the follow-up screening for cytological triage was low; only 46% of women with a positive HPV test result participated in the triage by Pap testing, 26% of whom underwent colposcopy (Louvanto et al. 2014).

The WOLPHSCREEN study from Germany reported that the CIN3+ rate was 0.87% among HPV-positive women in the first screening round (vs. 0.30% in study II and 0.47% in study III), while colposcopy referral was similar to our studies, at 3.9% (3.80% in study II and 4.38% in study III) (Luyten et al. 2014; Veijalainen et al. 2016; 2019).

An observational study from the UK demonstrated a higher rate of histological CIN2+ and CIN3+ at the baseline screening for the hrHPV group, 2.3% and 1.4%, respectively, in comparison with study II (0.6% and 0.3%, respectively) and study III (0.75% and 0.47%, respectively). Additionally, the colposcopy rate in the hrHPV-screened group at the baseline screening was also higher (6.8%) in comparison with our outcomes (Rebolj et al. 2019; Veijalainen et al. 2016; 2019). This difference can be explained by the fact that our attendees were HPV-test naïve, but they were not screening naïve, as they were screened with Pap test at age 30.

A population-based cohort study from the Netherlands reported that approximately 1.3 times more CIN2+ findings were found in the hrHPV-based primary screening program compared to the cytological-based screening program. Again, a higher rate of colposcopies was associated with the hrHPV-based program, as the number of colposcopies required to detect one CIN3+ lesion was 4.6 in the HPV-screened group and 3.0 in the cytology-screened group (Aitken et al. 2019).

6.2.5   The second HPV screening round

Study IV demonstrated that implementation of hrHPV primary screening leads to a decline in the total colposcopy rate by 29% from 4.0% to 2.9% in the second screening round among study participants. The study outcomes demonstrated a 53% (p = 0.003) reduction of performed colposcopies in women aged 35 and a 43% (p = 0.038) reduction in women aged 40. For the 45–55 age group, the reduction of the performed colposcopies did not reach a significance level. The lower colposcopy rate in the subsequent round can be explained by the high treatment rate of precancerous lesions in the first screening round and partly by the longer test interval prior to follow-up testing in the second round, as well as by the aging of the screened group with the decline in the number of women with persistent HPV infection. The

total decrease in colposcopy referrals was not as high as that reported in the randomized trials. Remarkable variations in study designs between RCTs and the present study can explain the differences in the results.

According to the NTCC Phase II study, the colposcopy referral decreased from 7.8% to 2.2% (Ronco et al. 2010). In this study, in the first screening round, all HPV-positive women were scheduled to colposcopy, while in the second screening round, women were tested only by cytology without HPV test and women with positive cytological tests were referred to colposcopy. In our study, in both screening rounds, all women with primary HPV+/LSIL+ and with persistent HPV-positive results at the follow-up screen were referred to colposcopy. The difference in the reason for colposcopy referrals between NTCC and our study explains the studies' outcomes. In the FOCAL trial, in the first screening round the rate of colposcopies was 5.6% and decreased to 1.2% in the second round. However, in the FOCAL study, the screened population was the same during both screening rounds, and the second screening took place after just 24 months, which makes the results incomparable with our study (Coldman et al. 2016). In the POBASCAM study, the colposcopy referrals declined in the subsequent round, from 2.3% to 1.3% (Bulkmans et al. 2007). The low colposcopy rate in the subsequent round of the HPV-screened group can be partly explained by the decrease in HPV prevalence with increasing age and slightly worse compliance in the subsequent round than in the baseline round (Bulkmans et al. 2007).

We concluded that our strategy to perform colposcopies on all women with persistent hrHPV can explain the high rate of colposcopies. It seems that we need a better triage for women with persistent high-risk papilloma virus infection, especially for younger women, to avoid unnecessary colposcopies.

Study IV demonstrated a declining trend of high-grade cervical lesions only in the second screening round, from 0.8% to 0.6% ($p = 0.220$). The findings from the randomized studies showed a significant decrease in precancerous lesions in the second round. In the NTCC Phase II trial, the number of histological CIN2+ lesions decreased from 0.60% to 0.03% in the second round (Murphy et al. 2012; Ronco et al. 2010). In the POBASCAM trial, histological CIN2+ lesions were found in 1.14% of women in the HPV-screened group in the first screening round and in 0.46% in the second round (Bulkmans et al. 2007; Murphy et al. 2012). Further, the SWEDESCREEN study demonstrated a decline in diagnosis of histological CIN2+ lesions in the second screening round, from 1.8% to 0.4% (Naucler et al. 2007; Murphy et al. 2012). In the FOCAL study, in the first screening round, the detection rate of CIN2+ was 1.53% and 0.2% in the second round (Coldman et al. 2016). The

fact that in the present study the decline of CIN2+ lesions (or histological HSIL) was not statistically significant in the second screening round may be related to the low case load, as the 35-year-old women had been screened cytologically five years earlier. We believe that a much larger population is required to demonstrate the decrease of CIN2+ lesions in subsequent screening rounds.

In our study, the proportion of women with HPV-positive test was 6.5% in the first screening round and 7.6% in the second screening round ($p = 0.02$). The age-specific analysis showed that the highest hrHPV prevalence was among the 35-year-old women, ranging from 9.7% to 12.0%. In older age groups, the HPV prevalence varied from 5% to 7% without any statistically significant variation between the different age groups in either screening round. Generally, our hrHPV positivity rates were well in line with the rates observed in randomized controlled trials: 5.8% of the study population in the NTCC study (age range 35–60 years) and 6.8% in ARTISTIC trial (age range 20–64 years) were hrHPV-positive (Patanwala et al. 2013; Kitchener et al. 2009; Ronco et al. 2010). Further, in the NTCC trial, the hrHPV-positive rate among women 25–34 years old was 11.7%, which is almost equal to what we observed among the 35-year-old women (Ronco et al. 2010; Patanwala et al. 2013). In the FOCAL study, 8.4% of women (age range 25–65) were reported as hrHPV-positive in the first screening round (Coldman et al. 2016).

6.3   Benefits and harms of implemented HPV screening

The purpose of any screening is to reduce the incidence and mortality of a specific disease. To achieve this, many factors need to be taken into consideration, such as validation of the screening test, determination of the age to start screening, when to end screening, the screening interval, effective screening services, follow-up services, systematic recalls in case of abnormal results, participation in the screening, etc. Any screening has both benefits and harms, and the benefits of the accepted screening should outweigh the harms.

Screening for cervical cancer is unique, as it does not aim to detect the early stages of cancer, but rather to find lesions that might progress to cancer. Due to the low incidence of invasive cervical cancer in the population previously screened, the efficacy of cervical cancer screening has been evaluated by rates of CIN3 and CIN3+ lesions in the second screening round. The outcomes from our studies demonstrated high sensitivity of HPV testing in detecting CIN2+ and CIN3+lesions at the first

screening round. The assessment of outcomes from the second screening round is not as straightforward. We could not distinguish histological CIN2+ and CIN3+ lesions among HSIL+ histological results in the second screening round due to the adapted use of the two-tiered terminology for reporting squamous intraepithelial lesions (as recommended by the WHO). The outcomes from the second screening round showed only a statistically insignificant decrease of HSIL+ lesions of 27% (p = 0.22), without any separate information on possible changes in the rates of CIN3+ lesions.

The policy to build a referral system for colposcopy based on persistent HPV infection seems to result in many colposcopies. In the Netherlands, HPV-positive women with normal cytology at baseline are triaged with repeat cytology at 6–12 months. Women with normal cytology at re-screening return to regular 5 years screening (Polman et al. 2019). This strategy for triage of HPV-positive women is based on the outcomes from the Dutch VUSA-Screen cohort study, which demonstrated a short-term reduction of CIN3+ from 5.2% in women HPV+/NILM at baseline to 1.6% in the women with NILM at re-screening at 6–12 months (Polman et al. 2019). This triage has high NPV (about 99%), high PPV (about 36%), and low colposcopy referral rates (about 39%). However, it should be noted that this strategy requires high-quality cytology.

 Alternative options have been suggested for the management of women with negative cytology at re-screening after HPV+/NILM. Acceptable options include referral for second testing after at least 12 months, return to routine screening, or referral to colposcopy, as in our study. Each country should use one of these options due to the absence of evidence on the best algorithm for HPV-positive women with normal cytology at baseline (European Guidelines 2008).

The detection of many precancerous lesions is associated with a high number of unnecessary colposcopies. According to our results, about five colposcopies needed to be performed to detect one HSIL+ lesion (Veijalainen et al. 2021). It should be noted that each diagnostic procedure increases the screening cost, including additional resources at different screening levels. Overdiagnosis causes psychological harm to participants with abnormal results. The diagnostic procedures and recommended treatments can cause immediate complications, such as bleeding, infection, physical discomfort, or later obstetrical complications, such as preterm delivery due to treatment (Arbyn 2008).

Our results are obviously preliminary. Although HPV screening detected a considerably higher number of precancerous lesions, we cannot judge its long-term effect on cervical cancer incidence, morbidity, and mortality. Conversely, we should

not disregard the possibility that the detection and treatment of moderately precancerous lesions may help in the reduction of more severe lesions. The hrHPV-based screening program in the Netherlands showed an increase in the diagnosis and treatment of precancerous lesions and false-positive referrals by 24% compared with cytological screening, the incidence of cervical cancer decreased by 1% and mortality by 4% (Jansen et al. 2021).

The reduction of colposcopies in the second round decreased the cost of the implemented screening. However, multiple factors affect the cost of screening. A cost-effectiveness analysis of the Finnish HPV screening program is currently lacking. The new Dutch hrHPV-based screening program was more cost-effective compared with cytology screening, with a 21% reduction in total cost (Jansen et al. 2021).

6.4   Strengths and limitations of the study

The most important strength of the study is the real-life setting. It is quite common that the outcomes of randomized trials will be diluted in real life when, for example, a screening method is implemented into routine practice. Even if some dilution was also seen in the present study, the findings were generally in line with the results of the randomized HPV screening trials.

We evaluated outcomes from a relatively large population of women who attended the organized cervical cancer screening program with Pap test and ART test with clinical validation for primary cervical cancer screening. The HPV-screened women in 2012 were comparable to the Pap-screened women in 2011 in terms of age, place of residence and screening laboratory. The attendance rates at the HPV screening were similar compared to cytological screening and were in concordance with the attendance rate of the whole country. The attendance rates in follow-up screenings were high.

Cytology and histopathology were investigated by experienced pathologists, and colposcopies were performed by trained gynecologists. Therefore, we can assume that our results are reliable. Our studies performed in a real-life setting showed the usefulness of the hrHPV test as a tool for cervical cancer screening and the benefit of hrHPV testing in triage of repeat ASC-US.

The present study has certain limitations. First, study I was retrospective and studies II–IV were prospective cohort studies, rather than randomized controlled

trials. Second, studies II, III and IV were limited by the partial heterogeneity of the study populations. In study II, we had a historical control representing the last totally cytologically screened population in Tampere. In study III, the hrHPV-screened group comprised residents in the city of Tampere, whereas the Pap group was represented by residents from municipalities in the Tampere Region. Although theoretically these two groups may behave differently, these municipalities do not represent truly rural areas, as a majority of their residents work or study in the city of Tampere. Moreover, the screening attendance rates were similar between the groups. In study IV, we followed the screening results of women aged 35–55 years in the first screening round and were 40–60 years old in the second screening round. The aging of the population inevitably led to a natural decline in the prevalence of HPV infections. Due to the migration of people, the screened population could be assumed to have partly changed. Third, in the second screening round of study IV, the re-screening visit was longer—that is, HPV-positive women with normal or borderline cytology were invited to re-screening after 12–16 months instead of 12 months, as was the case in the first screening round. The longer re-screening time could increase the clearance of HPV infections and could decrease the necessity of colposcopy. Finally, we are unaware of the incidence and prevalence of HPV infections and cervical (intraepithelial) neoplasia in those who did not undergo cervical screening.

7   FUTURE ASPECTS

In the first screening round, primary HPV screening with cytological triage resulted in a high colposcopy rate compared to conventional cytology screening. Nowadays, several additional tools have been proposed to increase the specificity for detection of cervical precancerous lesions among HPV-positive women. These include HPV partial genotyping, immunostaining for p16 with or without ki-67, detection of HPV E6 or E7 proteins and DNA methylation of host and viral genes.

HPV16 and HPV18 are linked to the highest risk of cervical cancer and its precursors. HPV partial genotyping is used as a triage in some countries outside of Europe. According to the Food and Drug Administration (FDA) guidelines for cervical screening in the United States, triage with HPV16/18 test is recommended as an option for women with an HPV-positive test result and normal cytology (Saslow et al. 2012). Women with a positive HPV test and negative cytology can undergo co-testing in 12 months or receive immediate HPV genotyping testing. Women who had positive HPV16 or HPV18 results should be immediately referred to colposcopy, while women who had negative HPV16/18 results should be referred to co-testing in 12 months. If co-testing is negative (HPV-negative and cytology NILM or ASC-US), the women should return to routine screening. Women with positive co-testing (HPV-positive or/and cytology LSIL+) should be recommend colposcopy. HPV genotyping can help in clinical management among women with abnormal cytology. For example, women with HSIL cytology and who are HPV16 positive have a 60% immediate risk of CIN3+; therefore, these women could be treated immediately without biopsies (Perkins et al. 2020).

In a Finnish population-based study, genotyping for HPV16, compared to cytology, was more successful in identifying women with CIN3+ at the threshold of LSIL+ (Leinonen et al. 2013). In another Finnish population-based study, HPV16/18 was present in 57% of CIN3 at the threshold of LSIL+ but only in 26% of CIN3 at the threshold of persistent hrHPV (Kares et al. 2019).

P16 and Ki67 are important biomarkers for detecting high-grade CIN among HPV-positive women. P16 is an important tumor suppressor protein that can induce cell-cycle arrest, while Ki-67 is a nuclear protein and an indicator of cell proliferation.

High expression of p16 and Ki-67 has been associated with high grade dysplasia. In the ATHENA study, dual-stained cytology for p16/Ki-67 was significantly more sensitive for triage of HPV-positive women in comparison with conventional cytology (74.9% vs. 51.9%, respectively, $p < 0.0001$), while specificity was comparable (74.1% vs. 75.0%, respectively, $p = 0.3198$) (Wright et al. 2017). The meta-analysis of the studies, which used double staining (p16/Ki67) for detecting CIN2+ lesions in triage of HPV-positive women, demonstrated a high sensitivity (81%; 95% CI: 72%–89%) and good specificity (72%; 95% CI: 62%–81%); for CIN3+, the sensitivity was 84% (95% CI: 73%–93%) and the specificity was 67% (95% CI: 54%–78%) (Arbyn, ESGO 2019).

Expression of viral oncogenes such as E6 and E7 is high among HPV-positive women with infections that put women at risk for cervical cancer (Cuzick et al. 2012). Clinical evaluation of E6/E8 assay with detection of oncoproteins from the eight most important carcinogenic types (HPV16, 18, 31, 33, 35, 45, 52, 58) showed 100% sensitivity, 85.9% specificity and 22.2% PPV in detecting CIN3+ among HPV-positive women. For detection of CIN2+ lesions, the assay with the eight-type human papillomavirus E6/E7 oncoprotein was less sensitive (67.7%) but more specific (89.5%), with a PPV of 46.7% (Rezhake et al. 2019).

In triage of hrHPV-positive women, a DNA methylation test (the S5 classifier) has shown a higher sensitivity in predicting CIN2+ compared to HPV 16/18 genotyping (Lorincz et al. 2016; Hernández-López et al. 2019). The S5 classifier is based on targeting the human suppressor gene EPB41L3 and the latest regions of HPV16, 18, 31 and 33. The S5 methylation test could reduce colposcopy referrals by 30%–50% in comparison with cytology and HPV16/18 genotyping triage without loss of sensitivity for CIN2+ and CIN3+ in hrHPV-positive women (Hernández-López et al. 2019). Furthermore, the DNA methylation status can predict the progression of untreated high-grade cervical lesions. In patients with histological CIN2 at baseline, the S5 classifier alone showed statistically high significance ($p = 0.001$) in predicting progression to CIN3+ versus regression to CIN1 (Louvanto et al. 2020).

An optimal triage of women with an HPV-positive test would help to recognize those who are at risk for developing cervical cancer and should be referred to immediate colposcopy. Moreover, it would guide practitioners in using the right strategy for the management of different contesting results and in avoiding overdiagnosis and overtreatment.

8   SUMMARY AND CONCLUSIONS

The main findings and conclusions of the presented work are the following:

1. Our retrospective study demonstrated the usefulness of hrHPV testing in the triage of repeat ASC-US cytology in women ≥30 years old. A high prevalence of hrHPV infections combined with a poor specificity of hrHPV tests for the outcomes of CIN2+ and CIN3+ among women with LSIL cytology limit the use of hrHPV testing as a triage of women with cytological LSIL.

2. The first year of implementation of the primary hrHPV screening in the setting of a routine organized cervical cancer screening resulted in a statistically significant increase by 2.06-fold in the rate of histological CIN2+ lesions, with an insignificant increase by 1.75-fold in the rate of histological CIN3+ lesions compared to conventional cytology. There was 1.84-fold significant increase in the rate of colposcopies in the hrHPV-screened group.

3. The first three years of implementation of the primary hrHPV screening with the Abbott RealTime HPV test resulted in significantly higher rates of both CIN2+ and CIN3+ precancerous lesions: by 2.45-folf and 2.70-fold, respectively. Again, HPV screening was associated with a twofold increase in the rate of colposcopies.

4. The second round of hrHPV primary screening demonstrated a statistically significant reduction in the rate of colposcopies by 29% from 4.0% to 2.9% with an insignificant decrease in the rate of high-grade cervical lesions by 27% from 0.8% to 0.6%, compared to the first screening round. The policy to refer all women with persistent HPV infection to colposcopy obviously resulted in an unnecessary high rate of colposcopies, with the risk of overdiagnosis and overtreatment. In future, a new screening algorithm with optimal triage of women with persistent HPV infection could help avoid the harms of HPV screening and increase the cost-effectiveness of hrHPV-based screening.

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PUBLICATION
I

High risk HPV testing in the triage of repeat ASC-US and LSIL

Veijalainen O, Tuomisaari S, Luukkaala T, Mäenpää J

AOGS ORIGINAL RESEARCH ARTICLE

High risk HPV testing in the triage of repeat ASC-US and LSIL

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Abstract

Introduction. The purpose of this study was to evaluate the real-life performance of high-risk (HR) HPV testing in the triage of repeat atypical squamous cells of undetermined significance (ASC-US) and low-grade squamous intraepithelial lesions (LSIL). *Material and methods.* In 2004–2012, 127 women with repeat ASC-US and 118 women with LSIL were triaged with HPV testing using either a Hybrid Capture 2® or Abbott RealTime® HR-HPV test. The patient charts were retrospectively reviewed for performance of the tests. *Results.* In the repeat ASC-US group, 40.9% of the women were positive for HR-HPV. The prevalence of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) was 8.7%. The sensitivity of the HR-HPV testing for detection of CIN2+ was 90.9% (95% Cl 58.7–98.5%), with a specificity of 63.8% (95%CI 54.4–72.5%). In women ≥30 years old, the specificity was 70.4%, whereas in younger women it was only 27.8%. The negative predictive value to predict CIN3 was 100% (95% CI 95.2–100.0%). Of the women with LSIL, 68.6% were positive for HR-HPV and the prevalence of CIN2+ was 14.4%. The sensitivity of HR-HPV testing for detection of CIN2+ was 94.1% (95% CI 71.2–99.0%), and the specificity 35.6% (95% CI 26.4–45.8%), respectively. The negative predictive value to predict CIN3 was 100% (95% CI 90.4–100.0%). *Conclusion.* HR-HPV testing seems to be beneficial in the triage of repeat ASC-US in women ≥30 years old, but a high prevalence of HR-HPV infection combined with poor specificity limit the use of HPV testing in the case of LSIL.

Abbreviations: ART, Abbott RealTime HR-HPV DNA test; ASC-US, atypical squamous cells of undetermined significance; CI, confidence interval; CIN, cervical intraepithelial neoplasia; HC2, Hybrid Capture II® HR-HPV DNA test; HR-HPV, high risk human papilloma virus; LSIL, low-grade squamous intraepithelial lesions; NPV, negative predictive value.

Introduction

In developed countries, the incidence of cervical cancer has decreased over the last decades due to organized screening programs (1). In Finland, an organized cytology-based cervical cancer screening program was established in the early 1960s. Since then, the cervical cancer incidence rate has decreased by 80%, with current incidence and mortality

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Key message

For at least 30-year-old women, high-risk HPV testing can be used in the triage of repeat ASC-US, but we do not recommend it for the triage of LSIL.

rates of 4/100 000 and 1/100 000, respectively. In 2011, cervical cancer was only the 17th most common cancer with 165 new cases (2). It is unlikely that any further marked improvement beyond these figures can be achieved with conventional screening. Quite recently, high-risk (HR) human papilloma virus (HPV) screening has been implemented for cervical cancer screening, either in the triage of equivocal cytological results (3) or as the primary screening method (4).

Since 2004, it has been our policy to test women aged at least 30 years for HR-HPV if they present with repeat atypical squamous cells of undetermined significance (ASC-US). Testing women with low-grade squamous intraepithelial lesions (LSIL) has been less consistent. The present study was undertaken to investigate the performance of this policy. However, as it turned out during acquisition of the data that the HR-HPV test had also been used in a considerable number of cases in women younger than 30 years of age, the analysis was extended to include these younger women.

Material and methods

The study was based on data extracted from the patient register of the Department of Obstetrics and Gynecology at Tampere University Hospital during 2004–2012. The charts of all women aged ≥16 years presenting with repeat ASC-US or LSIL were reviewed. Of note is that there was no formal study protocol but the patients reported here represent normal everyday patient flow at the outpatient clinic. We only excluded pregnant women ($n = 3$).

A total of 127 women of 130 with repeat ASC-US lesions (ASC-US group) and 118 women of 130 with LSIL lesions (LSIL group) were tested for HR-HPV. All these 245 women had been referred to the hospital for colposcopy. Twenty-two (17%) women with ASC-US cytology and 32 (27%) with LSIL cytology were <30 years old. The mean age in the repeat ASC-US group was 44.1 years [standard deviation (SD) 14.7 years] and in the LSIL group 40.4 years (SD 13.9), respectively.

The study was approved by the Ethics Committee of Tampere University Hospital (ETL R12228). No informed consent was obtained from the patients because this is a retrospective non-interventional study based on the routine patient records.

Cytology was performed by the conventional smear technique, and the results were reported using the New Bethesda System for cytologic diagnoses (5). Before 1 October 2010, HPV DNA was detected by Hybrid Capture II® (HC2) High-Risk HPV DNA Test (Qiagen, Gaithersburg, MD, USA) for 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Since 1

October 2010, HPV DNA was tested using the Abbott RealTime® (ART) HR-HPV assay (Abbott Molecular, Des Plaines, IL, USA) for 14 high risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) with concurrent distinction of HPV-16 and HPV-18 from 12 other HPV types. Previous studies have shown that HC2 and ART show a very similar performance (6), and we have grouped them together for the purposes of this study (see Tables S1–S4 for the separate results by HC2 and ART assays, respectively).

Statistical analysis

Sensitivity, specificity, negative predictive value (NPV) and positive predictive value with 95% confidence intervals were calculated by CONFIDENCE INTERVALS ANALYSIS – PROGRAM version 2.1.2 (University of Southampton, Southampton, UK). Differences between ASC-US and LSIL groups were tested by Pearson's chi-square test or Fisher's exact test. Statistical analyses were performed using IBM SPSS STATISTICS for Windows version 20.0 (released 2011) (IBM Corp., Armonk, NY, USA). *p*-values <0.05 were considered significant.

Results

HR-HPV DNA was found in 52 of 127 (40.9%) women in the ASC-US group and in 81 of 118 (68.6%) in the LSIL group ($p < 0.001$, Pearson's chi-square test). In the ASC-US group, HR-HPV was detected more frequently (77.3% vs. 33.3%) in young women aged 29 years or younger than in older women. A somewhat less profound tendency was seen in the case of LSIL (81.3% vs. 64.0%) (Table 1). Among women with repeat ASC-US the prevalence of histologically confirmed cervical intraepithelial neoplasia grade 2 or worse (CIN2+) was 8.7% (11 of 127 women). CIN2+ histology was detected in 18.2% of women 29 years or younger and in 6.7% of women 30 years or older ($p = 0.089$) (Table 1).

In the LSIL group, the prevalence of histologically confirmed CIN2+ was 14.4% (17 of 118 women). CIN2+ histology was detected in 25% of the women in the younger age group and in 10.5% of the women in the older group, respectively ($p = 0.151$) (Table 1).

In the ASC-US group only one false-negative HR-HPV result was observed among women with CIN2 histology, while all women with CIN3 were positive for HR-HPV (individual data on CIN3 not shown). The sensitivity of HR-HPV testing to detect CIN2+ and CIN3 was 90.9% (95% CI 58.7–98.5%) and 100.0% (95% CI 48.0–100.0%), and the specificity was 63.8% (95% CI 54.4–72.5%) and 61.5% (95% CI 52.2–70.1%), respectively. The positive predictive value (PPV) to predict CIN2+ and

Table 1. High risk HPV test (HC2 and ART) results and number of precancerous cervical lesions among women with ASC-US and LSIL cytology by age group

Age, years	Cytology	n	HPV+		HPV−		CIN2+, HPV+		CIN2+, HPV−	
			n	%	n	%	n	%	n	%
<30	ASC-US	22	17	77.3	5	22.7	4	18.2	0	0
	LSIL	32	26	81.2	6	18.8	8	25.0	0	0
≥30	ASC-US	105	35	33.3	70	66.7	6	16.7	1	1
	LSIL	86	55	64.0	31	36.0	8	9.3	1	1.2
30–39	ASC-US	29	16	55.2	13	44.8	4	13.8	0	0
	LSIL	25	22	88.0	3	12.0	0	0	0	0
40–49	ASC-US	26	6	23.1	20	76.9	0	0	1	3.8
	LSIL	25	16	64.0	9	36.0	7	28.0	0	0
50–59	ASC-US	32	5	15.6	27	84.4	1	3.1	0	0
	LSIL	23	12	52.2	11	47.8	1	4.3	0	0
>60	ASC-US	18	8	44.4	10	55.6	1	5.6	0	0
	LSIL	13	5	38.5	8	61.5	0	0	1	7.7

ART, Abbott RealTime® High Risk HPV test; ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HC2, Hybrid Capture 2® High Risk HPV test; HR HPV+, high-risk human papillomavirus positive; HR HPV−, high-risk human papillomavirus negative; n, number of cases.

CIN3 was 19.2% (95% CI 9.6–32.5%) and 9.6% (95% CI 3.2–21.0%) and the NPV was 98.7% (95%CI 92.8–99.8%) and 100.0% (95% CI 95.2–100.0%), for CIN2+ and CIN3, respectively (Table 1).

After the age of 30, the sensitivity of HR-HPV testing to detect CIN2+ among women with repeat ASC-US was 85.7% (95% CI 42.2–97.6%), and the specificity was 70.4% (95% CI 60.3–79.2%). PPV and NPV were 17.1% (95% CI 6.6–33.7%) and 98.6% (95% CI 92.3–99.8%), respectively (Table 2). Among women <30 years old the sensitivity of HPV testing to detect CIN2+ lesions was 100.0% (95% CI 40.2–100.0%), and the specificity was 27.8% (95% CI 9.8–53.5%).

Also in the LSIL group with CIN2+ histology, only one false-negative HR-HPV result was found and all women with CIN3 were positive for HR HPV. The sensitivity of HR-HPV testing to detect CIN2+ and CIN3 was 94.1% (95% CI 71.2–99.0%) and 100.0% (95% CI 62.9–100.0), and the specificity was 35.6% (95% CI 26.4–45.8%) and 33.6% (95% CI 24.9–43.3%), respectively. PPV for predicting CIN2+ and CIN3 was 19.8% (95% CI 11.7–30.1%) and 9.9% (95% CI 4.4–18.5%), and NPVs were 97.3% (95% CI 85.8–99.6%) and 100.0% (95% CI 90.4–100.0%), respectively (Table 1).

In the case of LSIL among women 30 years or older, the sensitivity of HR-HPV testing to detect CIN2+ was

Table 2. High risk HPV testing in the triage of repeat/persistent ASC-US and LSIL

Study	Triage group	n	Age	HPV test	Outcome	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Fait et al. (22)	Repeat ASC-US	226	28.2*	HC2	CIN2+	85.7	97.0	90.5	93.4
	Repeat LSIL	227	28.2*	HC2	CIN2+	88.2	94.7	84.5	96.1
Rebello et al. (8)	Persistent borderline	75	30**	HC2	CIN3	86.0	76.0	58.0	93.0
	Persistent mild dyskariosis	117	30**	HC2	CIN3	94.0	39.0	55.0	90.0
	Persistent both types	141	30**	HC2	CIN3	95.0	54.0	48.0	96.0
	Total subjects	166	<30 years	HC2	CIN3	94.0	33.0	51.0	89.0
		167	≥30 years	HC2	CIN3	91.0	72.0	55.0	96.0
Zielinski et al. (9)	Persistent borderline or mild dyskariosis	40	<40 years	HC2	CIN2+	90.0	30.0	30.0	90.0
		66	≥40 years	HC2	CIN2+	100.0	78.5	6.7	100.0
Present study	Repeat ASC-US	22	<30 years	ART and HC2	CIN2+	100.0	27.8	23.5	100.0
		105	≥30 years	ART and HC2	CIN2+	85.7	70.4	17.1	98.6
		Total 127	44.1*	ART and HC2	CIN2+	90.9	63.8	19.2	98.7

ART, Abbott RealTime High Risk HPV test; CIN, cervical intraepithelial neoplasia; HC2, Hybrid Capture 2 High Risk HPV test, HR HPV, high-risk human papillomavirus; n, number of cases; NPV, the negative predictive value; PPV, the positive predictive value.
*Mean, **Median.

88.9% (95% CI 51.7–98.2%), while the specificity was 39.0% (95% CI 28.1–50.8%). PPV and NPV were 14.6% (95% CI 6.5–26.7%) and 96.8% (95% CI 83.2–99.5%), respectively (Table 2). Among women aged 29 years and younger the sensitivity and specificity of HR-HPV testing to detect CIN2+ were 100.0% (95% CI 62.9–100.0%) and 25.0% (95% CI 9.8–46.7%), respectively.

Discussion

The risk of precancerous and cancerous lesions among women with ASC-US and even LSIL cytology is low. According to a meta-analysis performed by Arbyn et al. (3) the risk for CIN2+ among women with ASC-US cytology is 6–10%. For LSIL, the risk is somewhat higher, about 12–16% (10–12). In the present study, the prevalence of CIN2+ with repeat ASC-US and LSIL lesions was 8.7% and 14.4%, respectively.

Cytology has a low sensitivity (50–75%) to detect CIN2+, and several randomized trials and meta-analyses based on them have shown that HPV DNA testing improves detection of CIN2+ compared to mere cytology (4,10–13). According to the ALTS trial, the prevalence of oncogenic DNA among women with ASC-US cytology is 50.6% (95% CI 47.6–53.6%), which is considerably higher than the one reported by Stany et al. (40.8%; 95% CI 40.3–41.3%), probably reflecting the younger age of the participants in the ALTS trial (14,15). On average, the pooled positivity rate of HR HPV in women with ASC-US cytology was 43% (95% CI 40–46%) in the meta-analysis of Arbyn et al. in 2009 (16). In the present study, 40.9% of the women with repeat ASC-US tested positive for HR HPV. In a review of HPV testing for triage of minor cytological cervical lesions, the pooled absolute sensitivity of HC2 for CIN2+ with ASC-US cytology was 89.5% (95% CI 87.4–91.3%), with a specificity of 58.6% (95% CI 53.7–63.3%) (17). A meta-analysis of the diagnostic accuracy of the HC2 test compared with the APTIMA® HPV RNA test in triage of women with ASC-US showed that the pooled sensitivity and specificity of HC2 was 93.8% (95% CI 89.9–96.2%) and 46.8% (95% CI 40.6–53.1%), respectively, for CIN2 and 95.5% (95% CI 90.9–97.8%) and 44.9% (95% CI 33.3–57.1%), respectively, for CIN 3 (18). In the repeat ASC-US setting of the current study, the sensitivity (90.9%) of HR-HPV testing seems to be comparable, whereas the specificity (63.8%) appears to be better, especially in women more than 30 years old (70.4%).

A few previous studies have used HR-HPV testing in the triage of repeat cytological abnormalities (Table 2). The abnormalities study by Zielinski et al. (9) is perhaps the most comparable to our study of both the target pop-ulation and the end point. They had better sensitivity and specificity but poorer PPV in the older age group than we had in our repeat ASC-US group of at least 30-year-old women. The high NPV of HR-HPV testing is obvious across the studies, with only one study showing an NPV smaller than 90% in the age group of <30 years (8).

A slightly different approach was used by Siebers et al. (17). They evaluated the performance of a policy of triaging ASC-US and LSIL with either repeat cytology alone or combined with HR-HPV testing at 6 months. The additional HR-HPV testing reduced the proportion of repeat cytology at 18 months from 76.1 to 28.6%, and did not affect detection of CIN3, but slightly increased colposcopy rates and detection of CIN0-2. They concluded that additional HR-HPV testing would be beneficial in older women, especially if HR-HPV tests could be improved with more specific tests.

In the Norwegian Cervical Cancer Screening Program, a simultaneous HPV triage and repeat cytology was implemented in 2005 for women with unsatisfactory ASC-US and LSIL initial cytology. According to Nygard et al. (19), three different HPV tests were used, but 70% of women with ASC-US or LSIL repeat cytology were tested positive for HR-HPV with HC2. The three-year cumulative risk of CIN2+ was 43% in this group, whereas it was only 4% if HC2 was negative, further highlighting the high NPV of HR-HPV testing.

Due to its relatively high sensitivity (85.7%) and specificity (70.4%) in detecting CIN2+ lesions in the present study, HR-HPV seems to be useful in the triage of repeat ASC-US among women at least 30 years of age. Among women younger than 30 years old, the low specificity (27.8%) of HR-HPV testing limits its predictive value. However, our results in the younger age group should be interpreted with caution because a selection bias cannot be ruled out, due to the fact that the use of HR-HPV test was not systematic in this setting.

In the ALTS study, the rate of HR-HPV positivity in women with LSIL was 82.9% (95% CI 79.7–85.7%) (20). The earlier meta-analysis of Arbyn (21) reported that the pooled HR-HPV positivity was 76.6% (95% CI 70.9–82.3%), whereas, according to the more recent review, the HR-HPV positivity ranges from 55% to 89% (17). The present rate of 68.6% is closer to the lower limit of previously reported rates, probably reflecting the relatively high mean age (40.4 years) of our patients. The high prevalence of HR-HPV infection in women with LSIL leads to poor specificity of HR-HPV testing in the triage of LSIL seen in practically all studies (3,15,17,20,21, present study). It may be that the APTIMA® HPV RNA test would be better suited than DNA tests such as HC2 or ART for the triage of LSIL, because it seems to be more specific (18). However, a negative HR-HPV DNA test is

very reliable also in the case of LSIL, as its NPV was 100.0% in the present study for CIN3.

Conclusions

HR-HPV testing seems to be a good triage method for repeat ASC-US, at least among women aged 30 years and older. We do not recommend use of the HR-HPV test as a primary method in the triage of LSIL due to its poorer specificity in this setting.

Funding

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Hybrid Capture 2 High Risk HPV test (HC2) results and number of precancerous cervical lesions among women with ASC-US cytology by age group.

Table S2. Abbott RealTime High Risk HPV test (ART) results and number of precancerous cervical lesions among women with ASC-US cytology by age group.

Table S3. Hybrid Capture 2 High Risk HPV test (HC2) results and number of precancerous cervical lesions among women with LSIL cytology by age group.

Table S4. Abbott RealTime High Risk HPV test (ART) results and number of precancerous cervical lesions among women with LSIL cytology by age group.

PUBLICATION
II

Human papillomavirus test with cytology triage in organized screening for cervical cancer

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AOGS ORIGINAL RESEARCH ARTICLE

Human papillomavirus test with cytology triage in organized screening for cervical cancer

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Abstract

Introduction. In randomized studies, testing for high-risk (HR) human papillomavirus (hrHPV) has been more sensitive than conventional cytology in detecting cervical intraepithelial neoplasia (CIN). The aim of this study was to evaluate the performance of HPV testing in the setting of an organized routine screening program. *Material and methods.* Since 2012, 35- to 60-year-old women living in the city of Tampere have been screened with the Abbott RealTime hrHPV test. HPV-negative women are referred to the next screening round in five years. HPV-positive women are triaged with conventional cytology, and women with at least low-grade squamous intraepithelial lesion (LSIL$^+$) are referred to colposcopy. The remaining HPV-positive women are referred for re-testing after 12 months, and then all HPV-positive women are referred to colposcopy. The data from the last cohort with cytological screening (screened in 2011) is presented for comparison. *Results.* A total 5637 (70%) women attended the first round of HPV screening, and 369 were HPV-positive. Of them, 54 women LSIL$^+$ were referred to colposcopy, resulting in 16 CIN2$^+$ lesions found. Of the remaining HPV-positive women, 66% were still positive one year later, and were referred to colposcopy, with 18 additional CIN2$^+$ lesions found. The attendance rate to the last round of cytological screening was 71% (5814 women). Sixty-four women with LSIL$^+$ cytology were referred to colposcopy, and 11 CIN2$^+$ lesions were found. Of the 777 women with borderline cytology and scheduled for reflex screening in the following year, 109 (19%) had ASC-US$^+$, and 57 underwent colposcopy, resulting in six additional CIN2$^+$ lesions found. The total detection rate of CIN2$^+$ was significantly higher in the HPV-screened cohort (6.0/1000 vs. 2.9/1000, $p = 0.015$). However, the total colposcopy rate was 4% vs. 2%, respectively ($p < 0.001$). *Conclusion.* Human papillomavirus testing also seems to be more sensitive than cytology in detecting CIN2$^+$ lesions in the setting of a routine organized screening program, besides in the context of randomized trials. The problem of an increased colposcopy rate needs to be addressed in the future.

Abbreviations: AGC-FN, atypical glandular cells – favor neoplasia; AGC-NOS, atypical glandular cells, not otherwise specified; AIS, adenocarcinoma in situ; ASC-H, atypical squamous cells – cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of unknown significance; CIN, cervical intraepithelial neoplasia; hrHPV, high-risk human

papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; Pap, Papanicolaou; SCC, squamous cell carcinoma.

Introduction

Cervical cancer is the fourth most common cancer in women, with an estimated 528 000 new cases and 266 000 deaths worldwide in 2012 (1). In Finland, an organized population-based cervical cancer screening program was established in the early 1960s. In the Finnish program, the screening interval is five years; the screening starts at the age of 30 and the last invitation is sent to the women in the year when they turn 60. The trigger for a referral to colposcopy is cytological low-grade intraepithelial lesion (LSIL) or worse. Women with atypical squamous cells of unknown significance (ASC-US) or atypical glandular cells, not otherwise specified (AGC-NOS), are invited to participate in a reflex screening after 12 months. The screening program has reduced the incidence and mortality of cervical cancer by 80% from the baseline (2). In 2013, cervical cancer was only the 16th most common cancer among females in Finland. Still, each year in Finland, approximately 150 women develop cervical cancer and approximately 50–70 women die from this disease (3).

Persistent high-risk human papillomavirus (hrHPV) infection is necessary for the development of cervical precancerous and cancerous lesions (4). Based on this, primary hrHPV testing has emerged as an option for cervical cancer screening instead of cytology (5).

In 2012, hrHPV testing with cytology triage was introduced into routine cervical cancer screening for women aged 35–60 in Tampere (population 220 000). Women aged 30 are still being screened with conventional cytology only. These results are not presented here; this report concentrates on the first cohort of women routinely screened with the hrHPV test (the original screening round and the reflex screening round 12 months later) as a quality control measure. Corresponding data on the last cohort of 35 to 60-year-old women screened with conventional cytology was used as a comparator.

Material and methods

The report is based on a cohort of 11 451 women aged 35–60 attending the routine organized cervical cancer screening program from January 2011 to December 2012 in the city of Tampere (population 220 000). Eligible women were traced from the Population Information System by their year of birth. All women were invited to the screening via a letter within the regional screening program. The data from the last cytology-based screening round (year 2011) and the first HPV screening round (year 2012) are presented, extending to the reflex screening rounds 12 months after the original screening round. In both years, 30-year-old women were screened with conventional cytology and were excluded from the current analysis.

The cytological screening was based on the conventional Papanicolaou (Pap) smear. Pap samples were taken by trained nursing staff at the primary healthcare facilities of Fimlab Laboratories. The cytological samples were processed and analyzed at the laboratory of Tampere University Hospital (Fimlab) as part of routine practice. The Pap smear results were reported according to the New Bethesda System (6). Participants with normal cytology were referred to the next screening round in five years. Women with at least cytological LSIL (LSIL[+]) were referred to colposcopy. Women with borderline cytology (ASC-US or AGC-NOS) were scheduled to repeat the Pap smear at 12 months and were then referred to colposcopy if cytology turned out to be ASC-US[+] (Figure 1).

In the HPV-based screening, hrHPV DNA was tested by the Abbott RealTi*me* hrHPV test (RealTime; Abbott, Wiesbaden, Germany), according to the manufacturer's instructions. The RealTi*me* assay is an automated, qualitative, real-time PCR test for DNA detection of 12 high-risk human papillomavirus types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) with concurrent, separate detection of HPV16 and HPV18. The validity of the RealTi*me* HPV assay in detecting CIN2^{+} lesions in a screening setting has been confirmed in three randomized studies (7–9). The Abbott RT hrHPV test meets the criteria for use in primary cervical cancer screening (10). The results are reported here as hrHPV detected or hrHPV not detected. The detected results include positive HPV16, HPV18, non-HPV16/18 (a pooled result for other 12 HR HPV), and their combinations.

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Key Message

This study shows that HPV testing with cytology triage is applicable to organized cervical cancer screening.

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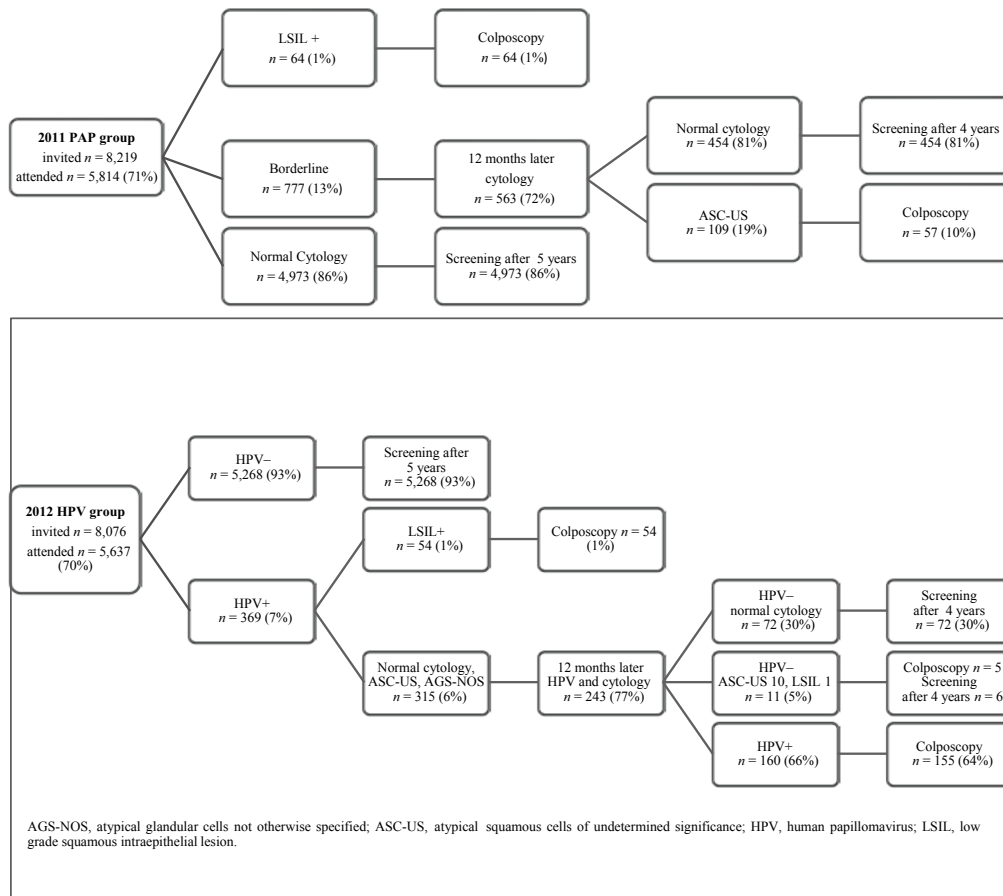
Figure 1. Screening algorithm. AGC-NOS, atypical glandular cells, not otherwise specified; ASC-US, atypical squamous cells of unknown significance; hrHPV, high-risk human papilloma; LSIL, low-grade squamous intraepithelial lesion; Pap, Papanicolaou; virus.

In the cohort screened with the HPV test, samples for both the HPV test and cytology were taken simultaneously by trained nursing staff at primary healthcare facilities of Fimlab Laboratories. The samples were processed and analyzed at the laboratory of Tampere University Hospital (Fimlab) as part of routine practice. HPV-negative women were scheduled for the next screening round in five years. The Pap test was evaluated only if the HPV test was positive. In addition, every 10th HPV test-negative sample was triaged with cytology as a quality-control measure. All women with HPV⁺/LSIL⁺ were referred to colposcopy. HPV-positive participants with normal or borderline cytology were scheduled to repeat both tests in 12 months. Then, all women with persistent HPV infection regardless of cytology were referred to colposcopy, as

well as those now HPV-negative but with repeat borderline cytology (or LSIL⁺). HPV-negative participants with previous normal cytology who now had borderline cytology were scheduled for the next screening round in four years. All women who had cleared their HPV infection and had normal cytology were scheduled for the next screening round in four years (Figure 1).

Colposcopies with biopsy and/or endocervical curettage were performed by experienced gynecologists in the outpatient department of Tampere Municipal Hospital as part of their routine practice.

This retrospective study was approved by the Ethics Committee of Tampere University Hospital (25 June 2013, Identification number ETL R13094). No informed consent was obtained from the participants because the

study describes normal routine screening practice and individual participants cannot be identified from this report.

Statistical analysis

The primary outcome measure was the detection of $CIN2^+$ lesions, including CIN2 and 3, AIS, and invasive cancer. Relative risks (RR) with 95% confidence intervals between screening groups were calculated using Confidence Interval Analysis (CIA) version 2.2.0 (http://www.som.soton.ac.uk/cia/). Categorical variables were tested by Pearson chi-square test or Fisher's exact test due to the too-small number of expected values. Statistical analyses were carried out using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA). Any p-values under 0.05 were considered statistically significant.

Results

In 2011, of the 8219 women invited, 5814 women (71%) participated in the cytology-based cervical cancer screening. However, the attendance rate varied from 60 to 78% in the different age groups (Table 1). In 2012, a total of 8076 women were invited to primary HPV screening, and 5637 participated (70%). Again, the attendance rate varied (61–75%) in the different age groups. The lowest attendance rate in both groups was among 35-year-old women, the attendance rate increasing significantly with age in both groups (p < 0.001). There were no differences in the pattern of attendance between the groups (p = 0.190).

The screening algorithm for the entire cohort is presented in Figure 1. In the cytologically screened group, only 86% (4973) of the attendees with normal cytology could be referred to the next screening round in five years, whereas the corresponding number (i.e. HPV test was negative) was 93% (5268) in the HPV group (p < 0.001).

Table 1. Age distribution and attendance rates in the PAP and the HPV group.

| Age, years | PAP group | | | HPV group | | |
| | Invited | Attended | | Invited | Attended | |
	n	n	%	n	n	%
35	1432	853	60	1409	862	61
40	1142	774	68	1092	684	63
45	1367	953	70	1398	960	69
50	1400	1049	75	1379	1034	75
55	1389	1027	74	1385	1031	74
60	1489	1158	78	1413	1066	75
Total	8219	5814	71	8076	5637	70

HPV, human papillomavirus; PAP, Papanicolaou.

Based on cytological abnormalities in the traditional screening group, a colposcopy was performed to 64 (1.1%) women (nine ASC-US, 22 LSIL, 12 cases of high-grade squamous intraepithelial lesion or HSIL, 14 cases of atypical squamous cells – cannot exclude HSIL or ASC-H, 3 AGC-NOS, and four cases of atypical glandular cells – favor neoplasia or AGC-FN). The primary colposcopy rate in the HPV group was also low, 54, or 0.9% (p = 0.450). Of these HPV-positive women, 34 had LSIL, seven ASC-H and 13 HSIL, respectively. Of note is that in both groups all referred women were compliant and underwent colposcopy. A total of 11 $CIN2^+$ lesions were found in the cytologically screened group and 16 $CIN2^+$ lesions in the HPV-screened group, respectively (Table 2, p = 0.300). Three cases of invasive squamous cell carcinoma (SCC) were found in the original screening round (one in a 50-year-old woman in the cytology group, and one each in 35- and 40-year-old women in the HPV group). In all three cases, the cytology was HSIL.

The percentage of women referred to the reflex screening after 12 months was significantly higher in the cytologically screened group than in the HPV-screened group (13% vs. 6%, respectively, p < 0.001). The respective attendance rates to the reflex screening were 72% vs. 77% (p = 0.111). In the cytology group, 57 (10%) of 563 underwent colposcopy (Figure 1). Colposcopically directed biopsies revealed six additional $CIN2^+$ lesions (Table 2).

In the HPV group, the reflex screening round showed that only 34% (83/243) had cleared their HPV infection (Figure 1). Of those, 72 attendees with normal cytology were referred to the next screening round in four years. However, 10 of the HPV-negative women had ASC-US on cytology, and one had LSIL. Six women with initial normal cytology and ASC-US at reflex screening were scheduled for the next screening round in four years. Four women with repeat ASC-US and the one with LSIL were referred to colposcopy. All of them had normal variants in colposcopically directed biopsies (three cases of normal histology and one each with inflammatory and koilocytotic changes). A total of 160 (attendance rate 97%) of 243 women were referred to colposcopy, based on a persistent positive HPV test (p < 0.001; Figure 1). Eighteen additional cases of $CIN2^+$ were found. The number of histological $CIN2^+$ lesions was significantly higher in the HPV-screened group than in the Pap group during the reflex-screening round, with an RR of 3.09 (95% CI 1.23–7.77) (Table 2). Two cases of invasive cancer were detected at the reflex screening: one carcinosarcoma (a 50-year-old woman in the cytologically screened group), and one squamous cell carcinoma (a 50-year-old woman in the HPV group).

Table 2. The total number of CIN2^{+} and CIN3^{+} (with subgroups AIS, and invasive cancer) among women aged 35–60 who attended cervical screening in 2011 and 2012 (with a reflex round in 2012 and 2013, respectively).

Histology	2011 PAP group (n = 5814)		2012 HPV group (n = 5637)		
	n	Detection rate per 1000 women	*n*	Detection rate per 1000 women	RR (95% CI)
CIN2[+]					
Original screening	11	1.9	16	2.8	1.50 (0.70–3.23)
Reflex screening	6	1.0	18	3.2	3.09 (1.23 –7.77)
Total	17	2.9	34	6.0	2.06 (1.15–3.69)
CIN3[+]					
Original screening	5	0.9	10	1.8	2.06 (0.70–6.03)
Reflex screening	5	0.9	7	1.2	1.44 (0.46–4.54)
Total	10	1.7	17	3.0	1.75 (0.80–3.82)
AIS					
Original screening	1	0.2	1	0.2	
Reflex screening	1	0.2	0	0.0	
Total	2	0.2	1	0.2	
Invasive cancer					
Original screening	1	0.2	2	0.4	
Reflex screening	1	0.2	1	0.2	
Total	2	0.3	3	0.5	

AIS, adenocarcinoma in situ; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus.
HPV group original screening, data 2012; HPV reflex screening, follow-up data after 12 months; PAP group original screening, data 2011; PAP group reflex screening, follow-up data after 12 months.

Overall, the first round of the primary HPV screening (the original and reflex rounds combined) identified 34 women with CIN2^{+} on histology (17 CIN2, 13 CIN3, three SCC and one adenocarcinoma in situ), which translates to a detection rate of 6.0/1000 CIN2^{+} lesions. Twenty-four of the CIN2^{+} lesions were detected in ≤45-year-old women. In comparison, the total number of CIN2^{+} lesions in the women attending the last conventional cytology-based screening (the original and reflex rounds combined) was 17 (seven CIN2, six CIN3, two AIS, one SCC, and one carcinosarcoma), or 3.0/1000 (Table 2). Again, most (11) lesions were detected in younger women. The difference in the detection rate of CIN2^{+} lesions between the HPV and PAP groups is statistically significant (RR 2.06; 95% CI 1.15–3.69, $p = 0.015$). Unfortunately, the low clearance rate of the HPV infection almost doubled the total number of colposcopies performed, 214 vs. 121, respectively ($p < 0.001$). Numerically also, the detection rate of CIN3^{+} lesions was higher in the HPV group, but in this case, the difference is not statistically significant ($p = 0.158$).

No cytological abnormalities were detected among the negative HPV-test samples during the quality-control measurements.

Discussion

A primary HPV screening, combined with cytology triage, within an organized regional screening program in round one showed a higher detection rate of histologically confirmed CIN2^{+} lesions in the HPV-screened cohort than in the last cohort screened with conventional cytology. The difference was in favor of HPV screening already in the original year, but was even more evident when the 12-month reflex rounds were also taken into account. For CIN3^{+}, the differences were not significant, probably due to a lower number of cases.

A problem in the Finnish cervical cancer screening program is the low attendance rate among younger screened cohorts. Of 30-year-old women, only half of the invited ones attend (Finnish Cancer Registry) (3). Of the age cohorts screened in Tampere in 2011 and 2012, the attendance rate among 35-year-old women was only 60%, whereas about 75% of women aged 50 or more attended, causing a potential bias in the detection rate of CIN2^{+} lesions. One possible explanation for the low attendance rate among younger women is a quite extensive opportunistic PAP screening in Finland (12) The fact that the present study concentrated only on 35-year-old and older women, combined with the skewed attendance rate, may explain why the detection rates of CIN2^{+} and CIN3^{+} lesions at the baseline screening rounds in both cohorts were lower than the rates in the UK ARTISTIC (11), Swedish SWEDESCREEN (13), Dutch POBASCAM (14), and Italian NTCC (15) randomized screening trials (Table 3). In those randomized trials, the mean age of the participants varied between 35 and 41 years (35 years for SWEDESCREEN, 39 years for ARTISTIC, 41 years for NTCC and POBASCAM), whereas in the present study the mean age of the participants was 50 years, which means that the participants of the randomized trials were prone to have a higher prevalence of transient HPV infections (16). Interestingly, a Finnish trial (17) which recruited 30 to 60-year-old women, established an almost identical rate of precancerous CIN3^{+} lesions in the HPV arm and conventional cytology arm as did our study (Table 3). Even this trial, although it also recruited

Table 3. Comparison with European randomized controlled trials.

		Number of women		Number of CIN2+			Number of CIN3+			Number of colposcopies		
	Age	HPV group	PAP group	HPV group (n/1000)	PAP group (n/1000)	RR (95% CI)	HPV group (n/1000)	PAP group (n/1000)	RR (95% CI)	HPV group (n/1000)	PAP group (n/1000)	RR (95% CI)
ARTISTIC (11)	20–64	18 386	6124	454 (24.7)	133 (21.7)	1.13 (0.94–1.37)	233 (12.7)	80 (13.1)	0.97 (0.75–1.25)	1247 (67.8)	320 (52.3)	1.28 (1.13–1.44)
SWEDESCREEN (13)	32–38	6238	6250	114 (18.3)	76 (12.1)	1.49 (1.12–1.99)	72 (11.5)	55 (8.8)	1.31 (0.92–1.85)	192 (30.8)	106 (17.0)	1.79 (1.42–2.26)
POBASCAM (14)	29–56	19 999	22 106	267 (13.4)	215 (10.7)	1.25 (1.05–1.50)	171 (8.6)	150 (6.8)	1.26 (1.01–1.56)	608 (30.4)	536 (24.2)	1.27 (1.13–1.42)
NTCC (15)	25–60	46 680	46 149	398 (8.5)	163 (3.5)	2.40 (2.00–2.88)	165 (3.5)	82 (1.8)	1.96 (1.51–2.55)	3825 (81.9)	1199 (26.0)	2.99 (2.81–3.19)
Finnish trial (17)	30–60	19 449	19 221	na	na	na	59 (3.0)	33 (1.7)	1.76 (1.15–2.70)	na	na	na
Present study	35–60	5637	5814	34 (6.0)	17 (2.9)	2.06 (1.15–3.68)	17 (3.0)	10 (1.7)	1.75 (0.80–3.82)	214 (38.0)	118 (20.3)	1.84 (1.48–2.30)

CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; na, not applicable; PAP, Papanicolaou; RR, relative risk.

30-year-old women, suffered from a low and skewed attendance rate of 67%. In all trials cited in Table 3, where the incidence rates of CIN2+ are given, HPV testing was more sensitive in detecting CIN2+ than cytology alone, with the exception of ARTISTIC (11). The lack of a significant difference in CIN3+ in our study may be explained by the rather low case load in our smaller population. On the other hand, the better sensitivity of the HPV test favors the detection of CIN2+ lesions. However, the poorer specificity makes it more challenging to demonstrate the superiority of the HPV test over cytology in detecting CIN3+ (18).

One invasive cancer was detected during the reflex screening both in the conventional and HPV screening groups: one carcinosarcoma and one squamous cell carcinoma, respectively. Both are considered to be failures of the primary screening. However, one may argue that if we had carried out conventional cytological screening in 2012 instead of HPV screening, the delay in SCC diagnosis would have been even greater, perhaps not occurring until the next screening round, because the cytology triage was negative and the referral to the reflex screening was based on HPV positivity only. The carcinosarcoma would perhaps have been missed with HPV screening also; on the other hand, cervical carcinosarcoma has been reported to be HPV-positive (19).

It is interesting that the greater sensitivity of HPV testing in the present study did not become evident until the reflex screening round. This may be explained by the fact that in HPV testing, the more sensitive test or HPV test was used for the primary screening, triaged by the more specific cytological test. This resulted in a similar rate of LSIL+ cytology, which was the trigger for direct colposcopy in both groups (Figure 1). In contrast, at the reflex screening 12 months later, HPV positivity in the HPV arm was an indication for colposcopy, resulting in a significantly higher detection rate of CIN2+ lesions (Table 2). However, the policy of retesting the HPV-positive women who did not have significant findings at cytology triage in the following year turned out to be a disadvantage in terms of an increased colposcopy rate. Although the initial colposcopy rate in the HPV cohort was not higher than in the cytology cohort, the fact that all HPV-positive women were referred to colposcopy at the reflex screening led to a significant increase in the total colposcopy rate compared with that in the cytology cohort, with an RR of 1.84 (95% CI 1.48–2.30). A similar tendency has been observed during the first screening round in the European randomized trials (Table 3). However, in those trials the significant reduction of CIN2+ in the HPV arm at the second screening round decreased the colposcopy rate in that arm and there was no difference in colposcopy rate between the HPV and Pap arms. Therefore, the ARTISTIC (11), SWEDESCREEN

(13), and PROBASCAM (14) trials did not detect an increase in the number of biopsies in the HPV arm during the entire period of observation, with a pooled ratio of 1.02 (95% CI 0.97–1.07) (16). It remains to be seen whether this type of phenomenon will also become evident in our HPV cohort at the second screening round. However, we are considering exploring available methods to increase the specificity of HPV testing such as HPV genotyping (20–22), p16/Ki-67 dual staining (23,24) and methylation marker (25).

In conclusion, primary hrHPV screening turned out to be more sensitive than conventional cytology in detecting CIN2$^+$ lesions in an organized cervical cancer screening program. A disadvantage is the increased rate of colposcopies at the reflex screening due to a high rate of persistent hrHPV infections.

Funding

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PUBLICATION
III

Implementation of HPV-based cervical cancer screening in an organized regional screening programme: 3 years of experience

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ORIGINAL ARTICLE

WILEY

Implementation of HPV-based cervical cancer screening in an organised regional screening programme: 3 years of experience

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Objective: The aim of this study was to evaluate the performance of human papillomavirus (HPV)-based screening in the framework of an organised cervical cancer screening programme.

Methods: A total of 46 708 women aged 35-60 years invited to the regional cervical cancer screening programme from 1 January 2012, to 31 December 2014, were enrolled. Overall, 17 770 women were screened by the Abbot RealTime hrHPV test with cytology triage and 15 605 were screened by conventional (Papanicolaou, Pap) cytology. In both groups, women with at least low-grade squamous intraepithelial lesions were referred directly for colposcopy, whereas HPV-positive women with borderline or normal cytology were invited to intensified screening in the following year. In the Pap group, the indication for intensified follow-up was borderline cytology.

Results: The attendance rate was similar in the HPV and Pap groups (72% and 71%, respectively). Overall, 6.0% of women in the HPV group vs 6.4% in the Pap group were referred to intensified follow-up (relative risk 0.94, 95% confidence interval [CI]: 0.87-1.03). At the index screening years, the relative sensitivity of the HPV test with cytology triage vs conventional screening was 1.64 (95% CI: 1.05-2.55) for CIN2+ and 2.06 (95% CI: 1.17-3.41) for CIN3+. The specificity of the hrHPV test with cytology triage for CIN2+ and CIN3+ was equal to that of the Pap screening (99.2% vs 99.2% for CIN2+ and 99.1% vs 99.1% for CIN3+).

Conclusions: Due to its high sensitivity and specificity, primary hrHPV testing with cytology triage seems to be acceptable for cervical cancer screening in an organised setting.

KEYWORDS
early detection of cancer, mass screening, Papanicolaou test, papillomavirus infections, squamous intraepithelial lesions of the cervix

1 | INTRODUCTION

Finland is a country with a low incidence of cervical cancer (5.82/100 000) due to an established, organised cytology-based screening programme since the 1960s. For example, 162 new cases were diagnosed in 2015. However, during the last 3 decades, the incidence and mortality of cervical cancer have not declined.[1]

Persistent high-risk human papillomavirus (hrHPV) infection is necessary for the development of practically all cervical cancers.[2] The fundamental role of hrHPV in the pathogenesis of precancerous

and cancerous cervical lesions has led to the development and implementation of HPV-based cervical cancer screening. Based on several randomised studies, it is now well established that hrHPV testing is more sensitive than cytology in detecting high-grade cervical intraepithelial neoplasia (CIN2+) or histological high-grade squamous intraepithelial lesions (HSILs) in women aged 30 years or older based on several randomised studies.[3-6] Pooled data from five recent randomised European trials have demonstrated a lower incidence of cervical cancer in women screened with HPV testing compared with cytology.[5,7,8] Consequently, the National Finnish Clinical Guidelines accepted hrHPV testing in 2016 as an equal alternative for Papanicolaou (Pap) smear in cervical cancer screening.[9] However, the guidelines in 2010 already allowed for a gradual implementation of HPV-based screening based on the promising results of early trials; thus, primary HPV screening was started in Tampere in 2012.

Our report on the first cohort (year 2012) screened with the hrHPV test exhibited a significantly increased number of CIN2+ lesions in HPV-screened women compared with the last Pap-screened cohort (in 2011). However, probably due to the low number of subjects with CIN3+, no significant difference in the detection rate of CIN3+ lesions[10] was noted. The purpose of the present study was to confirm these preliminary results in a larger screening population.

2 | METHODS

According to the Finnish Law, population-based screening for cervical cancer is offered to all women aged between 30 and 60 years.[11] Data concerning invitations, screening tests and test results are centrally collected and registered in the Mass Screening Registry, which was founded in 1968.[11] Local municipalities have the responsibility to organise cervical cancer screening. In the city of Tampere and its surroundings, the organiser is Fimlab Laboratories Ltd., a publicly owned laboratory service provider. Each woman is invited with a personal letter that describes the screening procedure. The screening interval is 5 years, but women with equivocal test results are invited to a risk group screening or intensified follow-up in the following year. Women with at least low-grade squamous intraepithelial lesions (LSIL+) are referred directly to colposcopy.

This observational and controlled but nonrandomised study is based on a cohort of 46 708 women aged 35-60 years invited by the municipalities to cervical cancer screening from 1 January 2012 to 31 December 2014 in the city of Tampere and its surroundings (total population 400 000). The outcomes included test results at the index screen and the results of the intensified follow-up after 12 months. The youngest invited women (the 30-year-old cohort) are not included in this report because this cohort was screened with the conventional Pap test only.

Primary hrHPV testing with cytology triage was implemented 1 January 2012 in the city of Tampere (the biggest municipality of the Pirkanmaa Region with a population of 220 000) and was offered to 24 692 women during the first 3 years or 2012-2014. The test used

was the Abbott RealTime test (RealTime; Abbott, Wiesbaden, Germany), a real-time polymerase chain reaction-based assay for concurrent detection and individual genotyping of HPV-16 and HPV-18 and the pooled detection of 12 additional hrHPV genotypes: HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-66 and HPV-68.[12] The test was launched on the European market in January 2009 and is currently used in many laboratories worldwide for routine HPV detection.[13,14]

Of the invited women, 17 770 (72%) attended the HPV screening with cytology triage (Figure 1). Cervical samples for hrHPV testing and cervico-vaginal samples for Pap smear were collected by a trained nurse at primary healthcare facilities of Fimlab Laboratories. Conventional cytology slides were analysed only for HPV-positive women. Pap smear results were reported using the Bethesda System.[15] All hrHPV-negative women were referred to the next screening round after 5 years, but each tenth HPV-negative test was triaged cytologically as a part of the quality assurance system. HPV-positive women with LSIL+ were referred to immediate colposcopy. The remaining HPV-positive women with normal or borderline (atypical glandular cells [ASC] of undetermined significance, AGC-not otherwise specified, regeneration) cytology were referred to the intensified follow-up after 12 months by both a new HPV test and cytology. At this risk group screening, all women with persistent HPV infection regardless of cytology result as well as HPV-negative women with cytological abnormalities (ASC-US or worse) were referred to colposcopy. Women with a negative HPV test and normal cytology were scheduled for the next routine screening round.

The conventional Pap screening was still used and offered in 2012 through 2014 to 22 016 women from seven municipalities surrounding the city of Tampere (Akaa, Kangasala, Nokia, Pirkkala, Lempäälä, Ylöjärvi, Valkeakoski), with a total population of 177 452; Figure 1). Altogether, 15 605 (71%) women attended the cytological screening. Participants with a normal Pap smear were referred to the next routine screening round after 5 years. The women with LSIL+ were immediately referred for colposcopy. Women with borderline cytology (ASC of undetermined significance, AGC-not otherwise specified, regeneration) were scheduled for risk group screening after 12 months with a repeat Pap smear. At the intensified follow-up, the indication for colposcopy was borderline or worse cytology, whereas women with normal test results were referred to the next routine screening round.

Colposcopies were performed by experienced gynaecologists as part of their normal routine practice in local hospitals.

The primary outcome measure was the relative sensitivity for colposcopically detected CIN2+ and CIN3+ lesions in the HPV and PAP groups, respectively. The relative sensitivity was calculated from the number of the CIN2+ and CIN3+ lesions divided by the number of women who participated in the screening. The specificity was calculated from the number of women with a negative screening test by dividing the number of screened women who did not have histologically confirmed CIN2+ or CIN3+ lesions. The positive predictive value (PPV) was calculated as the proportion of women with true
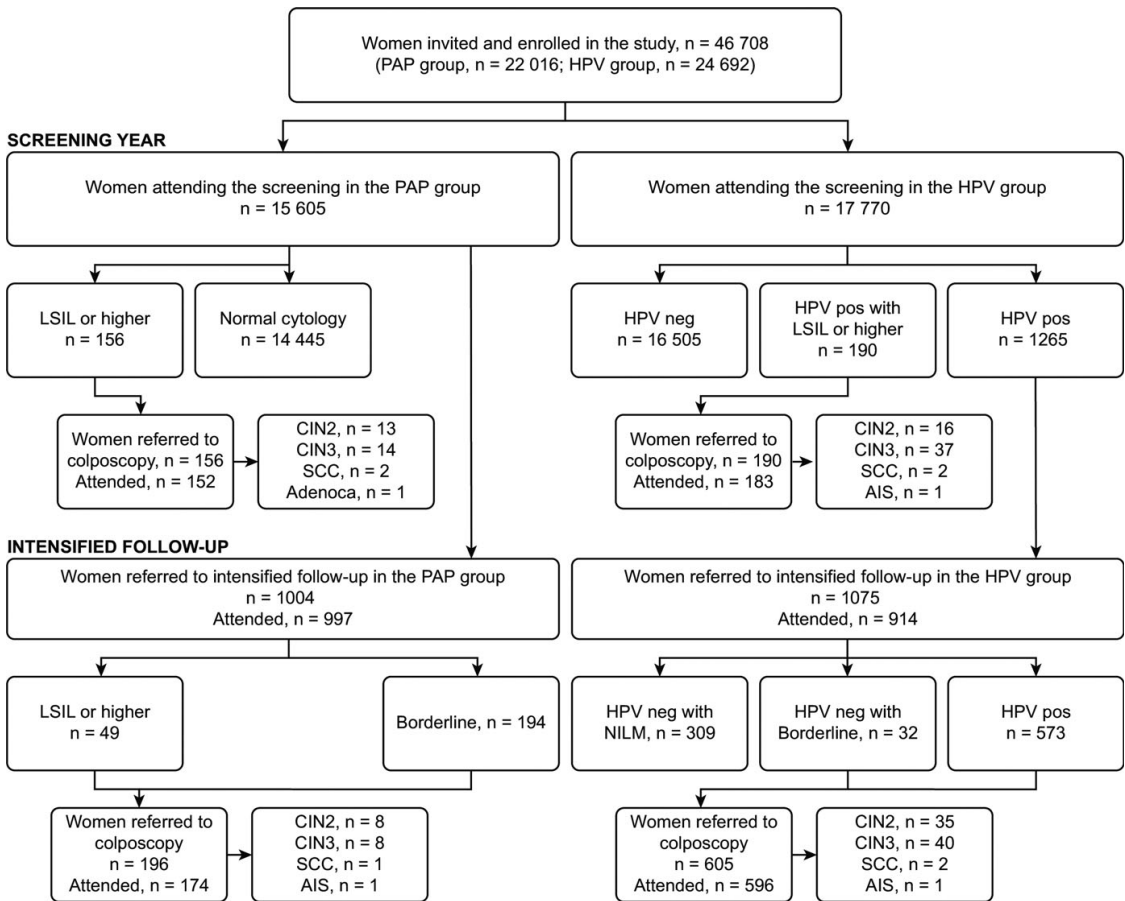
FIGURE 1 Flow chart of the study. AIS, adenocarcinoma in situ; Adenocarcinoma, adenocarcinoma of the cervix uteri; CIN, cervical intraepithelial neoplasia; HPV, human papillomaviruses; LSIL, low-grade squamous intraepithelial lesions; NILM, negative for intraepithelial lesion or malignancy; SCC, squamous cell carcinoma

positive histological results among women referred to colposcopy. The relative sensitivity, specificity and PPV were calculated for the index screening years. The relative sensitivity, specificity, PPV and relative risks (RR) with 95% confidence intervals (CIs) between screening groups were calculated using Confidence Interval Analysis (CIA) version 2.2.0 (http://www.som.soton.ac.uk/cia). Attendance rates were compared by Cochran-Armitage Trend Test using Stat-Xact version 4.0.1 (Cytel Software Corporation, ISBN 1-889592-05-6, Cytel, Inc., Cambridge, MA, USA). Differences between outcome groups and proportions of detected lesions were calculated by Pearson chi-square tests using IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp, Armonk, NY, USA). Any P-value $< .05$ was considered as statistically significant.

The study was conducted in accordance with the Helsinki Declaration and was approved by the Regional Ethics Committee of Tampere University Hospital (Identification number ETL R13094). No informed consent was obtained from the participants because the study describes normal routine screening practice and individual participants cannot be recognised from this report.

3 | RESULTS

Out of 24 692 women invited to the primary HPV screening with cytology triage and 22 016 women invited to the conventional Pap screening, 17 770 (72%) and 15 605 (71%), respectively, attended the screening (Figure 1).

As shown in Table 1, the average annual attendance rate in all age groups fluctuated from 61% to 78% with the lowest attendance rate noted among 35-year-old women in both the HPV and PAP groups. The attendance rate significantly increased with age in both groups ($P < .001$). No differences the pattern of attendance were noted between the groups (Table 1). The median age of the women in the HPV group and the PAP group was 45 years among the

TABLE 1 Age distribution, attendance rates, and CIN2+ and CIN3+ lesions in the Pap and the HPV groups

Age, y	Pap group				HPV group			
	Invited n	Attended n (%)	CIN2+ n	CIN3+ n	Invited n	Attended n (%)	CIN2+ n	CIN3+ n
35	3893	2383 (61%)	15	10	4497	2847 (63%)	41	28
40	3749	2519 (67%)	15	9	3456	2318 (67%)	26	16
45	3787	2638 (70%)	8	3	4007	2836 (71%)	22	12
50	3718	2793 (75%)	5	4	4224	3236 (77%)	20	15
55	3373	2540 (75%)	3	0	4222	3219 (76%)	12	6
60	3496	2732 (78%)	2	1	4286	3314 (77%)	13	6
Total	22 016	15 605 (71%)	48	27	24 692	17 770 (72%)	134	83

CIN, cervical intraepithelial neoplasia; HPV, human papillomaviruses; Pap, Papanicolaou

women invited and 50 years among those who attended, respectively.

A total of 16 505 (92.9%) women in the HPV-screened group with HPV-negative results were referred to the next screening round after 5 years compared with 14 445 (92.6%) women in the Pap group with normal cytology (P = .270). At baseline, 7.1% (1265 of 17 770) of the women in the HPV group were HPV positive and underwent cytology triage with outcomes of 1.1% (190 out of 17 770) LSIL+ cytology and 6.0% (1075 out of 17 770) borderline or normal cytology. In the screening year, the prevalence of LSIL+ cytology in the Pap group was equal to that of the HPV group (1%). However, the prevalence of LSIL+ among all HPV-positive women was 15.0% (190 out of 1265). Of note, no significant (worse than ASC-US) cytological abnormalities were detected among the HPV-negative women in the quality assurance programme.

Overall, 6.0% of women in the HPV screening group vs 6.4% of women in the Pap screening group were recommended for intensified follow-up at 12 months (RR: 0.94 [95% CI: 0.87-1.03]). Although the need for a colposcopy in the index screen years was similar in the HPV (1.0%) and the PAP (1.0%) groups (RR: 1.06 [95% CI: 0.85-1.31]), significantly more colposcopies were performed at the intensified follow-up in the HPV-screened group compared with the PAP-screened group (3.4% vs 1.1%, respectively) (RR: 2.99 [95% CI: 2.53-3.53]).

Despite a negative HPV tests at the risk group screening, none of the 32 women with cytological abnormalities exhibited CIN2 or any other significant finding in the colposcopically directed biopsies.

In both screening groups, younger attendees had/tended to have more lesions compared with older women (P < .001). There were slightly more CIN2+ and CIN3+ in the intensified follow-up groups in the HPV group, and vice versa in the PAP group (Table 2). In the index screening years, the prevalence rates of CIN2+ and CIN3+ in the HPV group were 0.32% and 0.26%, respectively, vs 0.19% and 0.11%, respectively, in the Pap group. At the reflex screening round, the prevalence rates of CIN2+ and CIN3+ in the HPV group were 0.44% and 0.24%, respectively, vs 0.12% and 0.06% in the PAP group. As the overall prevalence of CIN2+ and CIN3+ was 0.75% and 0.47%, respectively, in the HPV group vs 0.31% and 0.17%, respectively, in the PAP group. The relative sensitivity of the HPV test with cytology triage vs conventional cytology was 2.45 [95% CI: 1.76-3.41] for CIN2+ and 2.70 [95% CI: 1.75-4.16] for CIN3+. Overall, there were 25% (95% CI: 1.76-3.41) more CIN2+ and 27% (95% CI: 1.75-4.16) more CIN3+ lesions detected in the HPV-screened group compared with the PAP group (Table 2).

TABLE 2 The detection rate of CIN2 + and CIN3 + in the HPV and the Pap groups

Histology	HPV group (17 770) n	Pap group (15 605) n	RR (95% CI)
CIN2+			
Index screen	56	30	1.64 (1.05-2.55)
Intensified follow-up	78	18	3.80 (2.28-6.35)
Total	134	48	2.45 (1.76-3.41)
CIN3+			
Index screen	40 (2 SCC+1 AIS)	17 (2 SCC+1 AC)	2.06 (1.17-3.64)
Intensified follow-up	43 (2 SCC+1 AIS)	10 (1 SCC+1 AIS)	3.78 (1.90-7.51)
Total	83 (4 SCC+2 AIS)	27 (3 SCC+1 AC+1 AIS)	2.70 (1.75-4.16)

AC, adenocarcinoma of the cervix uteri; AIS, adenocarcinoma in situ; CI, confidence interval; CIN, cervical intraepithelial neoplasia; HPV, human papillomaviruses; IC, invasive cancer; Pap, Papanicolaou; RR, relative risk; SCC, squamous cell carcinoma.

	hrHPV (n = 17 770)				Pap (n = 15 605)			
	Sp	(95% CI)	PPV %	(95% CI)	Sp	(95% CI)	PPV %	(95% CI)
CIN2+	99.2	(98.8-99.6)	30.6	(24.4-37.6)	99.2	(98.7-99.5)	19.7	(14.2-26.8)
CIN3+	99.1	(98.6-99.5)	21.9	(16.4-28.4)	99.1	(98.6-99.4)	11.2	(7.0-17.2)

TABLE 3 Specificity and positive predictive value of hrHPV screening with cytology triage compared with the Pap screening for CIN2 + and CIN3 + histology at the index screen year

CIN, cervical intraepithelial neoplasia; hrHPV, high-risk human papillomavirus; Pap, Papanicolaou; PPV, positive predictive value; Sp, specificity.

The number of invasive cancers and adenocarcinoma in situ was quite small (Table 2). A total of four squamous cell carcinomas were detected in the HPV-screened group, whereas three squamous cell carcinomas and one adenocarcinoma of the cervix uteri were found in the cytological screening. None of the invasive cancers detected during the study in the cytologically or HPV-tested arms were HPV negative.

The specificity of the hrHPV test alone for CIN2+ and CIN3+ was clearly inferior to that of Pap screening (eg, the specificity of the hrHPV test alone for CIN2+ was 93.6% vs 99.2% in the Pap screening, and the specificity of the hrHPV test alone for CIN3+ was 93.3% vs 99.1% in the Pap group). However, no difference was noted in the specificity between the hrHPV test with cytology triage and Pap screening for CIN2+ and CIN3+ (99.2% vs 99.2% for CIN2+ and 99.1% vs 99.1% for CIN3+) (Table 3).

In the index screening years, PPV of the HPV test with cytology triage was significantly higher than PPV of the conventional cytology for CIN2+ and CIN3+ lesions. The relative PPV for hrHPV testing with cytology triage vs conventional cytology was 1.55 for CIN2 + (95% CI: 1.05-2.29, P = 0.027) and 1.95 for CIN3+ (95% CI: 1.16-3.30, P = 0.012).

4 | DISCUSSION

The findings of the present study confirmed the results of our previous report, which indicated that HPV-based screening works better than cytological screening in the setting of a routine cervical cancer screening programme as well as randomised trials.[10] However, in our preliminary study based on the 1-year screening cohorts, the difference between HPV-based screening and conventional screening was significant exclusively for CIN 2+ lesions. However, in this larger, 3-year screening cohort, the difference in favour of HPV screening was statistically significant for CIN2+ and CIN3+ lesions. Our study confirms that in the index screen years, the relative sensitivity of HPV testing with cytological triage vs conventional cytology is significantly increased for CIN2+ and CIN3+ lesions (RR: 1.64 with 95% CI: 1.05-2.55 and RR: 2.060 with 95% CI: 1.17-3.64, respectively).

Our relative sensitivity results for CIN2+ and CIN3+ lesions are not lower compared with other European studies. Meta-analysis of the eight trials in the developing and industrialised countries determined the relative sensitivity of HPV- vs cytology-based screening for CIN2+ 1.27 (95% CI: 1.06-1.52), ranging from 0.88 to 1.92, and for CIN3 + 1.14 (95%CI: 0.93-1.40), ranging from 0.83 to 2.06.[5] In the nonorganised screening programme in France, hrHPV test with cytology triage was more sensitive in the screening population.[16]

Although current World Health Organisation recommendations on the nomenclature of cervical precancerous lesions divide the lesions into two rather than three categories (histological LSIL and HSIL vs CIN1, CIN2 and CIN3),[17] CIN nomenclature is used in this study. The use of CIN terminology makes comparisons with other trials, including our own previous trial, easier to perform because large randomised trials also used the same nomenclature.[18-21] Moreover, the fact that histological HSIL combines CIN2 and CIN3 means that histological HSIL involves lesions with quite different tendencies for regression or progression.

The attendance rate in both screening groups was at the same level as the mean in Finland in general (approximately 70%).[22] The prevalence of hrHPV in our previous study[10] and the present study was 7.0% and 7.1%, respectively. Leinonen et al[23] reported 7.3% hrHPV prevalence in a cervical screening population in Finland. The overall high-risk HPV prevalence in women attending cervical screening in Europe varied from 2.2% to 15.7%.[24] According to Arbyn et al,[5] 10% (95% CI: 8%-12%) of the screened population was hr HPV-positive in European and North American studies.

The reliability of the Abbott RealTime hrHPV test for detecting CIN2+ lesions was investigated in four studies that evaluated the Abbott RealTime hr HPV test performance in primary cervical cancer screening settings in women 30 years and older.[13] According to these studies, the absolute clinical specificity of the Abbott RealTime hrHPV test alone for CIN2+ lesions ranged from 90.3% (95% CI: 89.4-91.1) to 93.5% (95% CI: 92.6-94.3).[12,25-27] In our study, the specificity of the Abbott RealTime hrHPV test alone for CIN2+ was similar at 93.6% (95% CI: 93.2-93.9). The randomised Finnish study reported almost the same outcomes for the specificity of a Hybrid Capture 2 assay alone for CIN2+ and CIN3+ lesions compared with the current study (93.0% and 93.6% for CIN2+ vs 92.7% and 93.3% for CIN3+, respectively).[23] Adding cytology triage to the primary hrHPV test increased the clinical specificity of hrHPV screening to the level of Pap screening at 99.2% [95% CI: 99.1-99.4] in the HPV group vs 99.2% [95% CI: 99.0-99.3] in the Pap group for CIN2+, which underscores the importance of cytology triage as an integral part of an organised screening programme even in the setting of HPV-based screening. Cytology results from hrHPV-positive women include an increased prevalence of abnormalities. However, reading becomes more difficult and specificity slightly decreases.[28,29]

The PPV of the Abbott RealTime hrHPV test with cytology triage was superior to conventional cytology for the detection of CIN2+ and CIN3+ lesions (30.6% vs 19.7% for CIN2 + , P = .024; 21.9% vs 11.2% for CIN3+, P = 0.011). Our results are comparable relative to those reported by Cuzick et al[26] in 2013. PPVs of the Abbott Real-Time hrHPV test for CIN2+ and CIN3+ among women referred to colposcopy were 33.9% (95% CI: 25.3-43.5) and 16.1% (95% CI: 9.8-24.2), respectively. The performance of the Abbott RealTime hrHPV test in the other population-based cervical cancer screening studies has demonstrated comparable PPVs for the detection of CIN2+ lesions at 15.6% (95% CI: 11.3-20.7)-20.6% (95% CI: 13.2-29.7).[27,30] According to these studies, the HPV test more correctly identifies women with precancerous lesions compared with conventional cytology. However, a problem is how to identify hrHPV-positive women who are at risk of having CIN2+ lesions or developing CIN2+ by the next screening round among all hrHPV-positive women.

Our policy to refer all women with persistent hrHPV infection at the reflex screening to colposcopy significantly increased (2.3-fold) the number of colposcopies compared with conventional screening (P < .0001). However, this effect was counterbalanced by the fact that the detection rate of CIN2+ lesions at the reflex screening compared with the original screening year was 3.8-fold increased (95% CI: 2.28-6.35) in the HPV group compared with the Pap group. Even the detection rate of CIN3+ lesions was 3.78-fold increased (95% CI: 1.90-7.51) in the HPV group compared with the Pap group. The current study clearly demonstrated the superiority of hrHPV testing compared with conventional cytology in identifying women with precancerous lesions but at the cost of more colposcopy referrals. It will be interesting to determine whether the colposcopy rate decreases when the HPV-screened cohorts attend the second HPV-based screening rounds. In previous randomised trials, excess colposcopy rates have generally plateaued in subsequent screening rounds.[7,21] In case this notion does not occur in our screening population, there are several options to increase the specificity of the hrHPV test. The use of HPV genotyping can improve the positive predictive value.[27,31,32] Stanczuk et al[33] suggested offering colposcopy to all HPV16/18-positive females and cytology/dual staining triage to other HPV genotypes. Leinonen et al[34] demonstrated that at the threshold of LSIL+, genotyping for HPV16 identified more women with CIN3+ compared with cytology. Another option to increase the specificity of the HPV test would be detection of mRNA for E6 and E7.[35] Moreover, immunostaining for molecular markers of proliferation or regulation of the cell cycle (ie, p16-INK4A, Ki-67) can be very sensitive for detecting of severe dysplasia.[36]

The current study has a few limitations. First, the study is retrospective rather than prospective and randomised. Second, the control group is not completely identical to the study group. Pap-screened women were residing in the surroundings of Tampere, whereas the HPV test was exclusively used in the city. However, there are also important similarities between the groups. The groups exhibited an identical screening history, and the attendance rate was similar. Moreover, the majority of women who live in the surrounding areas commute to work in the city. Finally, the study population was too small and the follow-up was too short to allow conclusions regarding any beneficial effect in the detection of early invasive cancer. The strength of the study is that it represents a real-world experience and confirms that the findings obtained in randomised trials are relevant and reproducible in routine screening practice.

5 | CONCLUSION

The study provides confirmatory data on the superiority of primary hrHPV screening with cytology triage compared with conventional screening in the setting of a routine organised cervical cancer screening programme.

CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest regarding this article.

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PUBLICATION
IV

Primary HPV screening for cervical cancer: Results after two screening rounds in a regional screening program in Finland

Veijalainen O, Kaares S, Kotaniemi-Talonen L, Kujala P, Vuento R, Luukkaala T, Kholová I, Mäenpää J

ORIGINAL ARTICLE

Primary HPV screening for cervical cancer: Results after two screening rounds in a regional screening program in Finland

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Abstract

Introduction: Since 2012, cervical cancer screening has been conducted with a primary high-risk human papillomavirus (hrHPV) test and conventional cytology triage in the city of Tampere, Finland. The women who were screened with the hrHPV test in 2012 were invited to participate in the second screening round in 2017. The aim of the present report was to compare the number of colposcopy referrals and the number of histological high-grade squamous intraepithelial lesion (HSIL)+ (cervical intraepithelial neoplasia [CIN2+]) lesions between the first and second screening rounds of women of a specific age group who were screened twice with the hrHPV test.

Material and methods: The primary hrHPV test used was the RealTime hrHPV PCR assay by Abbott. Women with a positive hrHPV test and cytology triage equal to or worse than low-grade squamous intraepithelial lesion or atypical glandular cells, favor neoplasia, were directly referred to colposcopy, whereas hrHPV-positive women with a negative or equivocal cytology triage were re-screened after approximately 12-16 months. hrHPV-negative women were scheduled for re-screening after 5 years. The present report focuses on the cohort of women who were screened twice with the hrHPV test, who were 35-55 years old in 2012, and 40-60 years old in 2017.

Results: In all, 8076 women were invited for HPV screening in 2012 and 8331 women were invited for the second round 5 years later, with attendance rates of 70% and 71%, respectively. Of the women who were screened in 2012, 4571 (69%) belonged to the 35- to 55-year age cohort. In 2017, 4807 (73%) of the women aged 40-60 years participated in the screening. In this cohort, 185 (4.0%) colposcopies were performed in the first screening round, compared with 139 (2.9%) in the second round, and the colposcopy rate was 29% smaller in the second round ($P = .002$). The number of histological HSIL+ cases was 38 (0.8%) during the first screening round and 29 (0.6%) during the second round ($P = .220$).

Conclusions: In the setting of routine organized cervical cancer screening, the initially high colposcopy rate associated with primary HPV screening seems to level off at the second screening round in women who were screened twice with an hrHPV test.

KEYWORDS

cervix, gyneoncology, human papillomavirus, human papillomavirus screening, infections, neoplasia, women's health issues

1 | INTRODUCTION

Genital human papillomavirus (HPV) infections are very common, with a lifetime infection rate of approximately 80% in the adult population.[1] The majority of HPV infections are transitory and clear spontaneously in 12-24 months.[2] The rate of clearance depends on many factors, such as virus type, host immunological factors, and possible co-infections.[3] Persistent infection with an oncogenic HPV type (high-risk HPV [hrHPV]) may lead to the development of a precancerous lesion and, eventually, to invasive cervical cancer.[2,4,5] Further, hrHPV DNA is known to be detectable in at least 99.7% of cervical cancer specimens.[4,6] Hence, hrHPV testing has become widely studied as a primary screening test, although specific screening protocols have varied. Many studies have applied cytology as a co-test or triage test.[7-10]

Testing for hrHPV is currently accepted as the primary screening modality for cervical cancer by the World Health Organization and the European Guidelines for Quality Assurance for Cervical Cancer Screening.[11,12] In 2012, the American Cancer Society guidelines for the early detection of cervical cancer recommended HPV DNA testing every 5 years, with cytology co-testing as the primary cervical screening test for women who are 30-65 years old.[13]

Primary HPV screening with cytology triage was first implemented in the Finnish cervical screening program in January 2012. Initially, the HPV screening protocol was followed only in the city of Tampere (population approximately 240 000). The results of the first screening round have been reported previously in detail.[14] In brief, the results showed that primary HPV screening doubled the number of histological high-grade squamous intraepithelial lesion (HSIL)+ (cervical intraepithelial neoplasia [CIN]2+) lesions that were identified, compared with cytological screening (6.0/1000 vs 2.9/1000). However, the number of colposcopy referrals also increased significantly (38.0/1000 vs 20.8/1000).[14,15] In previous randomized screening studies, the increase in the number of colposcopies observed during the first screening round has been found to level off in the second round.[7,16-18]

We followed a cohort of women aged 35-55 years who took part in the first HPV screening round in 2012 until the second screening round in 2017, when these women were 40-60 years old. The aim of the study was to compare the colposcopy rate and the rate of histological HSIL+ lesions identified in the respective screening rounds.

Key Message

In the setting of routine organized cervical cancer screening, the initially high colposcopy rate associated with primary HPV screening levels off at the second screening round in women who were screened twice with an hrHPV test.

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2 | MATERIAL AND METHODS

The study is based on individual-level data from 16 407 invitations among women aged 35-60 years who were scheduled for routine cervical cancer screening with hrHPV test (5-yearly interval) in Tampere, Finland, in 2012 and 2017. Personal ID codes of the target-aged women who were invited to the routine cervical screening program at the time of interest were collected from the National Population Information System by their year of birth. For these women, we collected information on attendance and results of two screening rounds: the first round starting in January 2012 and the second round in January 2017. The study algorithm is shown in Figure 1. Thirty-year-old women were excluded from the study, because at the time of the study, they were still screened only by conventional cytology. The focus of the present report is on women who were screened twice with the primary HPV test, who were 35-55 years old in 2012 and 40-60 years old in 2017.

Samples were taken by trained nursing staff at the primary health-care facilities of Fimlab Laboratories. Specimens for both the hrHPV test and cytological smear were collected at the same visit. The hrHPV test was first analyzed in the laboratory. Women with a negative hrHPV test result were considered screening negatives, and they were referred for screening in the second round, 5 years later. However, 10% of the smears of hrHPV-negative women were initially evaluated as part of our quality control program. As no false-negative hrHPV tests were found, this policy was abandoned after a couple of years.[14] In women with a positive hrHPV test, cytological smears were always analyzed. Women with positive hrHPV test results and low-grade squamous intraepithelial lesion (LSIL)+ (LSIL, atypical squamous cells, cannot exclude HSIL [ASC-H], HSIL, atypical glandular cells, favor neoplasia [AGC-FN], or more severe) cytology were directly referred to colposcopy, which was performed
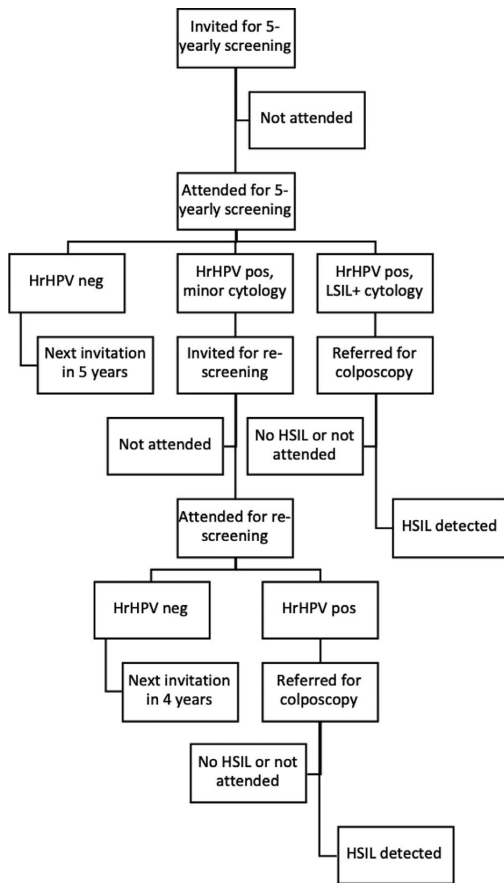
FIGURE 1 Screening algorithm for a screening round. Abbreviations: hrHPV, high-risk human papillomavirus; LSIL+, low-grade squamous intraepithelial lesion or worse; HSIL, high-grade squamous intraepithelial lesion

within 1 month for ASC-H, cytological HSIL, or AGC-FN cytology and within 6 months for LSIL cytology. The rest of the women who were hrHPV-positive, that is, women with normal or atypical squamous cells of undetermined significance (ASC-US)/ atypical glandular cells not otherwise specified (AGC-NOS) cytology, were referred for re-screening with an hrHPV test and cytology, on an average of 12-16 months later, as well as further colposcopy if the HPV test was repeatedly positive, regardless of the cytology result. All women who had cleared their hrHPV infection at the re-screening returned for routine screenings every 5 years, which took place over the next 4 years (Figure 1). The same schedule and procedures were applied at both screening rounds. However, during the first screening round, the re-screening interval was an average of 4 months shorter (range 7-16 months) than during the second round (range 12-22 months). In both rounds, the colposcopy procedures were performed by gynecologists at regional hospitals as part of their normal clinical practice. One year since obtaining the screening result was allowed for getting

confirmation about a performed colposcopy. The data cut-off was October 2019.

During the first screening round, one reminder was sent to women who did not respond to the primary invitation, whereas two reminders were sent during the second round. Upon referral to colposcopy clinics, the clinics took care of all further actions, including possible reminders to non-responding women.

We used the Abbott RealTime hrHPV PCR assay (RealTime; Abbott) for the DNA detection of 14 hrHPV types (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). Only HPV16 and HPV18 were reported separately; the other detected types were reported as grouped into the category "other hrHPV type." The hrHPV test that was used was launched in January 2009 in European markets and met the specific clinical performance criteria for use in primary cervical cancer screening in women 30 years and older.[13,19]

Cytological screening was based on conventional smears according to the Bethesda 2014 system.[20] The histopathological results for squamous intraepithelial lesions were reported using the latest World Health Organization Classification of Tumors of Female Reproductive Organs, in which CIN grade 2 and 3 lesions were grouped together as histological HSIL.[21]

2.1 | Statistical analyses

Relative risks (RR) and differences between percentages with 95% CIs between screening groups were determined using the R statistical software package (version 3.6.2).[22] Differences with *P*-values <.05 were considered statistically significant.

2.2 | Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Regional Ethics Committee of the Expert Responsibility Area of Tampere University Hospital on 25 June 2013 (Identification number ETL R13094). No individual informed consent was required, as the study was conducted within routine screening practice, and individual participant data were not recognizable.

3 | RESULTS

A total of 8076 women aged 35-60 years were invited to undergo HPV screening in Tampere in 2012 and 8331 women in 2017, with participation rates of 70% and 71%, respectively (Table 1). Of the women who participated in 2012, 4571 (69%) were 35-55 years old. In 2017, 4807 (73%) women who were 40-60 years of age attended the program (see Supporting Information Figure S1).

In 2012, the proportion of women with a detectable hrHPV infection was 321/4571 (7.0%), while in 2017, the proportion

TABLE 1 Invitations and attendance to primary human papillomavirus screening in 2012 and 2017

Age (y) at 5-yearly screening	Year 2012		Year 2017	
	Invited n	Attended % (n)	Invited n	Attended % (n)
35	1409	61 (862)	1702	64 (1095)
40	1092	63 (684)	1327	71 (944)
45	1398	69 (960)	1112	69 (766)
50	1379	75 (1034)	1394	72 (1000)
55	1385	74 (1031)	1391	75 (1049)
60	1413	75 (1066)	1405	75 (1048)
All ages	8076	70 (5637)	8331	71 (5902)

was 321/4807 (6.7%) (P = .51; Figure S1). Of the hrHPV-positive women, 49/321 (15.3%) and 36/321 (11.2%) had a positive cytology triage test and were directly referred to colposcopy in 2012 and 2017, respectively (P = .103; Table S1, Figure S1). The rest were scheduled to undergo repeat testing the following year (Figure 1). If a woman remained hrHPV-positive the following year, she was referred to colposcopy, regardless of the cytology triage result. The number of women who were repeatedly positive for HPV was 141/321 (43.9%) and 110/321 (34.3%) in the first and second screening rounds, respectively (P = .02; Figure S1). Due to incomplete compliance, only 136 and 103 women eventually had a colposcopy performed in 2013 and

2018, respectively (P = .013; Supporting Information Table S2 and Figure S1).

The total number of colposcopies performed in the first screening round was 185 (4% of the participating women), while 139 (2.9%) were performed in the second screening round (Table 2). There was a 29% decline in the number of colposcopies, and this difference was statistically significant (P = .002). The rates of colposcopies performed at the index screen and at re-screening in each screening round are shown in Supporting Information (Tables S1 and S2). In both rounds, more colposcopy referrals were made in the re-screening year than in the original screening year, which is based on repeated hrHPV positivity with normal or mildly abnormal cytology.

The details of the number of histological HSIL+ lesions are given in Table 3 and Supporting Information (Tables S3 and S4). The total number of histological HSIL+ lesions was 38 (0.8%) in the first screening round and 29 (0.6%) in the second round. Although there was a 27% decline, this difference was not statistically significant (P = .220; Table 3), nor was the number of colposcopies needed to detect one histological HSIL+ lesion (P = .761; Table 4).

4 | DISCUSSION

The most important finding of this retrospective cohort study was that the initially high colposcopy rate declined significantly in age cohorts screened twice with an hrHPV test in the framework of a routine organized screening program. Our results are in this respect in

TABLE 2 Rate and relative risk of performed colposcopies, in the first and the second screening rounds in women who were 35-55 years old in 2012 and 40-60 years old in 2017

Age (y) 2012	Colposcopy rate in the first screening round, n (%)	Age (y) 2017	Colposcopy rate in the second screening round, n (%)	RR (95% CI) 2017 vs 2012	P value
35	43 (5.0)	40	22 (2.3)	0.47 (0.28-0.77)	.003
40	33 (4.8)	45	21 (2.7)	0.57 (0.33-0.97)	.038
45	32 (3.3)	50	30 (3.0)	0.90 (0.55-1.47)	.700
50	39 (3.8)	55	34 (3.2)	0.86 (0.55-1.35)	.552
55	38 (3.7)	60	32 (3.1)	0.83 (0.52-1.32)	.466
Total	185 (4.0)	Total	139 (2.9)	0.71 (0.57-0.89)	.002

Abbreviations: RR, relative risk.

TABLE 3 Rate and relative risk of histological HSIL+ in the first and the second screening rounds in women who were 35-55 years old in 2012 and 40-60 years old in 2017

Age (y) 2012	Histological HSIL+ rate in the first screening round, n (%)	Age (y) 2017	Histological HSIL+ rate in the second screening round, n (%)	RR (95% CI) 2017 vs 2012	P value
35	16 (1.9)	40	10 (1.1)	0.57 (0.26-1.25)	.170
40	5 (0.7)	45	4 (0.5)	0.71 (0.19-2.65)	.743
45	7 (0.7)	50	5 (0.5)	0.69 (0.22-2.15)	.573
50	6 (0.6)	55	3 (0.3)	0.49 (0.12-1.97)	.339
55	4 (0.4)	60	7 (0.7)	1.72 (0.50-5.86)	.548
Total	38 (0.8)	Total	29 (0.6)	0.73 (0.45-1.17)	.220

Abbreviations: HSIL, high-grade squamous intraepithelial lesion; RR, relative risk.

TABLE 4 Number of colposcopies required to detect one histological HSIL+ in the first and the second screening rounds

Age (y) in 2012	Number of colposcopies required to detect one histological HSIL+, first screening round	Age (y) in 2017	Number of colposcopies required to detect one histological HSIL+, second screening round	RR (95% CI) 2012 vs 2017	P value
35	2.7	40	2.2	0.74 (0.42-1.31)	.319
40	6.6	45	5.3	0.72 (0.50-1.03)	.079
45	4.6	50	6.0	1.25 (0.85-1.84)	.284
50	6.5	55	11.3	1.71 (1.26-2.32)	<.001
55	9.5	60	4.8	0.50 (0.35-0.70)	<.001
Total	4.9	Total	4.8	0.93 (0.62-1.39)	.761

Abbreviations: HSIL, high-grade squamous intraepithelial lesion; RR, relative risk.

TABLE 5 Colposcopy referrals and histological HSIL+ (CIN2+) lesion rates; results of the second screening round compared with the first screening round in five randomized trials and two observational studies

Study (Reference)	Colposcopy rate		Histological HSIL+ rate	
	First round (%)	Second round (%)	First round (%)	Second round (%)
NTCC Phase II [17,23]	7.8	2.2	0.55	0.03
POBASCAM [18]	2.3	1.3	1.14	0.46
SWEDESCREEN [17,24]	n.a.	n.a.	1.8	0.4
ARTISTIC [25,26]	6.8	2.4	2.46	0.56
FOCAL [16]	5.6	1.27	1.53	0.2
WOLPHSCREEN [27]	n.a.	n.a.	1.3	0.43
Current study	4.0	2.9	0.8	0.6

Abbreviations: CIN, cervical intraepithelial neoplasia; HSIL, high-grade squamous intraepithelial lesion; n.a., not available.

line with those of five randomized trials (Table 5)[16-18,23-26]. However, the reduced colposcopy rate was not accompanied by a significant decline in the number of histological HSIL+ lesions that were detected. Possible explanations for this apparent discrepancy include a chance finding only, as the number of histological HSIL+ lesions is quite low. Furthermore, our cohort was not screening naive in 2012 but was HPV test naive; the women had been cytologically screened when they were 30 years old. This likely contributes to the relatively low number of lesions. We found also one observational study from Germany, which was conducted in the framework of a routine local cervical cancer screening program using Papanicolaou smear and Hybrid Capture 2 (HC2) HPV co-testing, and which extended over more than one screening round (Table 5).[27] Unfortunately, no data on colposcopy referrals were given, but both the histological HSIL+ and invasive cervical cancer rates declined significantly in the later screening rounds compared with the first round.

The 70% screening attendance rate is in line with the general cervical cancer screening rate in Finland (71.62% in 2018) but somewhat lower than in other Nordic countries (Sweden 84.02%, Norway 79.74%, and Iceland 79.54% in 2018).[28,29] It is unfortunate that the attendance rate is poor, especially among the youngest age groups, where the prevalence of HPV infections is highest.

The major strength of this study is that it provides real-life experience in HPV-based screening and confirms the results of randomized

trials regarding the colposcopy rate decline in cohorts screened twice with an hrHPV test. However, this is not immediately reflected in the colposcopy rate in general, as the peak prevalence of HPV infections is in the youngest age groups. If we compare the whole screening population in 2012 with that in 2017, the colposcopy rates are similar, because of the high colposcopy rate in 35-year-old women (Supporting Information Table S5). This could probably be avoided using better triage methods than cytology, for example, methylation panels.[30] However, if cytology triage only continues, this problem will probably continue for approximately 10 years, until the National HPV Vaccination Program will hopefully change the situation. The program started in 2013, since which time, girls aged 11-12 years have been vaccinated with the bivalent vaccine. Unfortunately, vaccine coverage is only about 70%. However, the recent inclusion of boys in the Vaccination Program is likely to increase coverage in the future.

The weaknesses of the study include the lack of an appropriate control group. In a randomized setting, one can control for the effect of time and aging, as one can compare 35-year-old women to 35-year-old women, 40-year-old to 40-year-old, etc. In the present setting, the effect of aging is prone to explain at least partly the decrease seen in the colposcopy rate. Another problem is that as the attendance rate was only 70%, we do not know how many women attended both screening rounds de facto, and this may dilute the effect of repeated screening. One factor that contributes to the

lower colposcopy rate during the second round may be the longer interval between the index screen and re-testing. Nevertheless, as the factors discussed above are prone to bias the results more towards diluting them, rather than exaggerating them, it is quite safe to state that the rate of colposcopies declines by approximately 30% in age cohorts tested twice over two consecutive HPV screening rounds.

5 | CONCLUSION

This real-life experience implies that, as in randomized trials, the initially high colposcopy rate typical to primary HPV screening, levels off in age cohorts screened twice with hrHPV test also in the setting of a routine organized screening program.

CONFLICT OF INTEREST
None.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.