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Prevalence of HPV infection and use of HPV test in cervical cancer screening:

Randomised evaluation within the organised cervical cancer screening programme in Finland

Maarit Leinonen

Academic Dissertation

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“If you´re willing to change the world
Let love be your energy”

Robbie Williams

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1 ABSTRACT

A randomised trial on alternative screening methods implemented in the organised cervical cancer screening programme has been running in Finland since 1999. In this trial, screening with an automation assisted cytology and screening with a primary HPV DNA test (the latter since 2003) is compared to screening with a conventional cytology (Pap test). The ultimate aim of the trial is to assess the incidence of cervical precancerous lesions and cancer following the initial screening visit. This setting enables to evaluate the effectiveness of screening using different screening modalities.

The aim of this study was to study the prevalence of the carcinogenic cervical HPV infection in Finland and to evaluate the use of an HPV DNA test in cervical cancer screening as a primary screening test. A cytology triage followed for women who were found to be HPV DNA-positive. HPV DNA-positive women were then referred to colposcopy based on the cytology triage result, similarly as in the conventional protocol.

Performance and validity of a primary HPV DNA test with cytology triage in comparison with a conventional cytology was evaluated both in a cross-sectional and in a prospective setting. Screening methods were compared by measuring following cross-sectional parameters at the initial (index) screening: test positivity, recommendation to intensive screening, referral to colposcopy and histological detection rates. We also estimated relative sensitivity, relative specificity and positive predictive values of the screening methods for different histological outcomes. Effects of screening methods on the burden of precancerous lesions and cancer were studied also in a longitudinal follow-up design. We analysed hazard ratios between study arms and cumulative hazards of cervical lesion for the different histological outcomes from the prospective data.

The majority of the cervical precancerous lesions in Finland are currently detected outside the national programme. Thus, the effectiveness of screening in the Finnish female population cannot be completely evaluated from the cervical lesions detected only within the screening programme. Therefore, we used data from the screening register and appended it by retrieving cervical lesions also from the Finnish Cancer Registry and from the Care Registers for Social Welfare and Health Care (formerly the Finnish Hospital Discharge Register, HDR) maintained by the THL. These registers included also lesions that were detected outside the national screening programme.

Our study showed a similar inverse relationship between the prevalence of carcinogenic HPV infection (high-risk HPV, hrHPV) and age reported in other developed countries. Age was found to be a strong determinant of hrHPV infection. Other significant risk factors included marital status and a previous hysterectomy.

Prevalence rate of any hrHPV infection reflects the background risk for cervical cancer. It was at the same level, or at least it was not markedly lower than in other European countries. This indicates that the low burden of cervical cancer is due to the health care actions including free public screenings within the organised programme.

Type-specific HPV prevalence was somewhat lower than suggested by international meta-analyses. The most common hrHPV type was HPV 16 followed by 31 and 52. The distribution of the hrHPV types in Finland was closest to reports from Eastern Europe suggesting that HPV types found in Finland are consistent with a regional HPV type

distribution in the world. HPV 16 attributed one fifth of the referrals to colposcopy but caused clearly more than half of the most severe cervical precancerous lesions and cancers (CIN 3+).

At the index screen visit, there were equal numbers of colposcopies in both study arms. However, screening by the HPV DNA test detected significantly more mild and moderate cervical lesions (CIN 1 and CIN 2) in comparison with screening by the cytology. The relative specificity and the positive predictive value (PPV) of the HPV DNA test alone were inferior to cytology. However, the relative specificity of the HPV DNA test with cytology triage was similar and even slightly better than that of cytology among women 35 years and older. The PPVs of the HPV DNA test with cytology triage were consistently better than those of conventional screening. Recommendations to intensive screening were made more often in the HPV than in the conventional arm. This was mainly due to the low age of the screening population (<35 years).

During one screening round of five years, the HPV test identified women at risk for severe cervical precancerous lesion or cancer (CIN 3+) markedly better than cytology. The cumulative detection rates of cervical lesions over one screening round showed that a very few cases of CIN 3 or AIS were diagnosed later than three and a half years after an invitation to an HPV screening arm among women aged 35 years and older. On the contrary, there was a rather constant increase in the detection of CIN 3 or AIS in the conventional screening arm between the years of two and five following the initial screening. This difference between the screening arms suggests an opportunity for earlier diagnosis of high-grade cervical lesions if HPV DNA test would be used used as a routine screening test.

After a negative HPV DNA test result (92% of the screened), there were substantially less CIN 3+ cases detected than after a normal result in cytology (93% of the screened) at the index screen visit. This indicates that HPV-based screening better identifies women with whom an extended screening interval would be safe. This could potentially reduce the screening demand for a large group of women and thus result in significant cost savings.

In a population-based screening programme, most women are healthy. Also, CIN 3 and cancer are rare outcomes in well-screened populations. This necessitates a careful balance between sensitivity and specificity of the screening tests. On the other hand, when the outcome is rare, there is then less potential for new interventions to improve prevention. Thus, more aggressive protocols might be warranted to increase the effectiveness of screening. An important issue in HPV screening is how to manage HPV-positive women. Intensive surveillance with frequent colposcopies may easily result in overdiagnosis and overtreatment of cervical lesions. This in turn may have economical and psychosocial consequences and result in morbidity for women of reproductive age.

When the HPV DNA test is considered as a measure for routine use, then age groups and screening intervals need to be carefully selected. This applies particularly to the algorithm that follows a positive HPV DNA test result. HPV testing should only be done within the organised screening programme whilst a gradual implementation of HPV screening in other regions in Finland would be preferred. This allows for systematical evaluation of possible adverse effects and the effectiveness of screening.

2 FINNISH SUMMARY

Kohdunkaulan syöpää ehkäisevän väestöseulonnan yhteydessä on Uudenmaan alueella vuodesta 1999 lähtien arvioitu uusia kohdunkaulan seulontamenetelmiä satunnaistetussa tutkimusasetelmassa. Tutkimuksessa verrataan automaatioavusteista Papa-seulontaa sekä HPV-testiin perustuvaa seulontaa (jälkimmäinen vuodesta 2003), perinteiseen Papa-seulontaan. Tutkimuksen tarkoituksena on seurata seulonnan jälkeistä esiaste- ja syöpäilmaantuvuutta sekä syöpäkuolleisuutta satunnaistetuissa seulontaryhmissä. Näin voidaan arvioida seulonnan vaikuttavuutta eri seulontamenetelmillä sekä havaita mahdolliset erot ryhmien välillä.

Tämän väitöskirjatyön tavoitteena oli selvittää kohdunkaulan karsinogeenisten HPV-infektioiden vallitsevuus (prevalenssi) suomalaisessa seulontaväestössä sekä tutkia HPV-DNA-testin käyttöä ensisijaisena seulontatestinä. Positiivisen HPV-DNA-testin jälkeen naisille tehtiin sytologinen jatkotesti (triage), jonka perusteella naiset ohjattiin tarvittaessa jatkotutkimukseen (kolposkopia ja koepalat) kuten perinteisessä seulontahaarassa.

HPV-seulonnan toimivuutta ja validisuutta arvioitiin sekä poikkileikkaus- että pitkittäisasetelmassa verraten perinteiseen Papa-seulontaan. Poikkileikkausasetelmassa seulontamenetelmiä arvioitiin mittaamalla testipositiivisten määriä, seuranta- ja jatkotutkimussuositusten määriä sekä löydösmääriä ja niiden riskisuhteita ensimmäisen seulontakäynnin yhteydessä. Lisäksi arvioitiin seulontatestin suhteellinen herkkyys, suhteellinen tarkkuus ja positiivinen ennustearvo eritasoisille päätemuuttujille. Pitkittäisasetelmassa tutkittiin kohdunkaulan syövän esiasteiden ja syöpien hasardisuhde (hazard ratio) tutkimushaarojen välillä sekä esiasteiden ja syöpien hasardikertymä (cumulative hazard) tutkimushaaroissa.

Suurin osa Suomessa todetuista kohdunkaulan syövän esiasteista todetaan seulontaohjelman ulkopuolella. Tällöin seulontaohjelman vaikuttavuutta kohdeväestössä ei voida arvioida yksistään seulontaohjelmassa todettujen esiaste- ja syöpälöydösten perusteella. Väitöstutkimuksessa on yhdistetty jouokkotarkastusrekisterin seulontatietoihin tiedot syöpärekisterin ja THL:n Hoitoilmoitusrekisterin esiaste- ja syöpälöydöksistä seulontakierroksen aikana sekä seulontaohjelman ulkopuolella.

Tutkimus osoitti, että karsinogeenisten HPV-infektioiden vallitsevuus vähenee naisten ikääntyessä. Iän lisäksi muita merkittäviä HPV-infektion ristitekijöitä olivat siviilisääty ja aiemmin tehty kohdunpoisto.

Karsinogeenisten HPV infektioiden vallitsevuus ryhmänä vastaa yleisesti eurooppalaista tasoa, tai ei ole ainakaan merkittävästi matalampi. Tämä viittaa siihen, että kohdunkaulan syövän taustariski Suomessa on samanlainen kuin muualla Euroopassa. Suomen alhainen kohdunkaulan syöpäilmaantuvuus ja syöpäkuolleisuus ovat siten todennäköisimmin seurausta terveydenhuollon toiminnasta, mikä tukee organisoidun väestöpohjaisen seulontaohjelman merkitystä.

HPV-infektioiden tyyppikohtainen vallitsevuus oli jonkin verran alhaisempi kuin kansainvälisten meta-analyysien perusteella olisi voinut olettaa. Yleisimmät virustyypit olivat HPV 16, 31 ja 52. Virustyyppien jakauma Suomessa muistutti eniten Itä-Euroopasta julkaistuja raportteja, joten Suomen yleisimmät virustyypit vastaavat maantieteellistä virustyyppien jakaumaa maailmassa. HPV 16 oli syynä viidesosaan kolposkopialähetteistä

mutta aiheutti selvästi yli puolet kohdunkaulan syövän vaikeista esiasteista ja syövistä (CIN 3+).

Ensimmäisellä seulontakerralla molemmissa seulontaryhmissä tehtiin saman verran kolposkopioita, mutta HPV-testiin perustuva seulonta löysi poikkileikkausasetelmassa merkittävästi enemmän vain lieviä ja keskivaikeita (CIN 1 ja CIN 2) syövän esiasteita Papa-seulontaan verrattuna. Pelkän HPV-DNA-testin tarkkuus ja positiivinen ennustearvo olivat huonompia kuin sytologian. HPV-DNA-testi ja sytologinen jatkotesti olivat kuitenkin poikkileikkausasetelmassa tarkkuudeltaan perinteisen Papa-seulonnan veroiset ja yli 35-vuotiailla naisilla jopa sitä parempi. HPV-DNA-testin ja sytologisen jatkotestin positiivinen ennustearvo oli kauttaaltaan perinteistä Papa-seulontaa parempi. HPV-seulonnasta aiheutui merkittävästi enemmän seurantasuosituksia kuin perinteisestä Papa-seulonnasta, mikä johtui pääasiallisesti alle 35-vuotiaiden naisten seulonnasta.

Ensimmäisen viisivuotisen seulontakierroksen aikana HPV-seulontaryhmässä todettiin merkittävästi enemmän vaikeita esiasteita ja syöpiä (CIN 3+). Hasardikertymät osoittivat, että HPV-seulontaryhmässä ei juurikaan todettu uusia CIN 3+ tapauksia enää 3,5 vuoden jälkeen seulontakutsun ajankohdasta. Perinteisessä seulontaryhmässä sen sijaan CIN 3+ hasardikertymä kasvoi suhteellisen tasaisesti toisen ja viiden vuoden välillä seuranta-aikana. Tämä voi viitata vahvan esiasteen varhaisempaan diagnostiikkaan, mikäli seulonnassa käytettäisiin HPV-DNA-testiä. Toisaalta negatiivisen HPV-testin jälkeen seuranta-aikana todettiin merkittävästi vähemmän CIN 3+ löydöksiä kuin normaalin Papa-testituloksen jälkeen. Negatiivinen HPV-testitulos, jonka 92% seulottavista naisista sai, antoi siis paremman suojan tulevaisuuden vaikeaa esiastetta ja syöpää vastaan. Tämä mahdollistaisi pidemmän seulontavälin niille useimmille naisille, joiden HPV-testi on negatiivinen ja säästäisi todennäköisesti seulontaohjelman kustannuksia.

Väestöpohjaisessa seulonnassa valtaosa tutkittavista naisista on terveitä ja CIN 3 ja syöpä ovat harvinaisia päätemuuttujia hyvinseulotussa väestössä. Tämä edellyttää seulontatestiltä hyvää herkkyyden ja tarkkuuden tasapainoa. Toisaalta, tällöin myös millä tahansa uudella seulontamenetelmällä on vain rajallinen mahdollisuus lisätä seulonnan vaikuttavuutta, ja se voi vaatia aggressiivisempia toimintamalleja. HPV-seulonnan osalta merkittävä kysymys on kuinka tulisi suhtautua HPV-positiivisiin naisiin. Liian intensiivinen seuranta ja / tai kolposkopiat voivat johtaa kohdunkaulan syövän esiasteiden ylidiagnostiikkaan ja ylihoitamiseen. Tällä puolestaan voi olla taloudellisia ja psykososiaalisia vaikutuksia. Esiastehoidot saattavat lisätä myös ennenaikaisen synnytyksen riskiä, mikä on otettava huomioon fertiili-ikäisiä naisia seulottaessa.

Kun HPV-testi otetaan rutiinikäyttöön, tulee kiinnittää huomiota siihen minkä ikäisiä naisia sillä seulotaan ja kuinka tiheästi. Erityisen huomionarvoista on seurantasuositusten ja jatkotutkimusten intensiteetti positiivisen HPV-testituloksen jälkeen. Tämän vuoksi olisi suositeltavaa asteittain laajentaa HPV-seulontaa Suomessa organisoidussa seulontaohjelmassa, jolloin seulonnan vaikuttavuuden ja mahdollisten haittavaikutusten arviointi on asianmukaisesti järjestetty.

3 LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by their Roman numerals (I-IV).

I Leinonen M, Kotaniemi-Talonen L, Anttila A, Dyba T, Tarkkanen J, and Nieminen P (2008) Prevalence of oncogenic human papillomavirus infection in an organised screening population in Finland. Int J Cancer 123: 1344-9.

II Leinonen M, Nieminen P, Kotaniemi-Talonen L, Malila N, Tarkkanen J, Laurila P, and Anttila A (2010) Age-specific evaluation of primary human papillomavirus screening versus conventional cytology in a randomized setting. J Natl Cancer Inst 101: 1612-23.

III Leinonen MK, Nieminen P, Lönnberg S, Malila N, Hakama M, Pokhrel A, Laurila P, Tarkkanen J, and Anttila A (2012) Detection rates of precancerous and cancerous cervical lesions with one screening round of primary human papillomavirus DNA testing: prospective randomised trial in Finland. BMJ 345:e7789.

IV Leinonen MK, Anttila A, Malila N, Dillner J, Forslund O, and Nieminen P (2013) Type- and age-specific distribution of human papillomavirus in women attending cervical cancer screening in Finland. Br J Cancer 2013 1-10 | doi: 10.1038/bjc.2013.647.

4 ABBREVIATIONS

AGC-FN	Atypical glandular cells, favour neoplasia
AGC-NOS	Atypical glandular cells not otherwise specified
AIS	Adenocarcinoma in situ
ASCUS	Atypical squamous cells of undetermined significance
ASCUS+	Atypical squamous cells of undetermined significance or worse
ASC-H	Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion
BMD	Borderline or mild dysplasia
CADM1	Cell adhesion molecule 1
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CIN 1-3	Cervical intraepithelial neoplasia, grade 1-3
CIN 1+	Cervical intraepithelial neoplasia grade 1 or more severe lesion
CIN 2+	Cervical intraepithelial neoplasia grade 2 or more severe lesion
CIN 3+	Cervical intraepithelial neoplasia grade 3 or cancer
CIN NOS	Cervical intraepithelial neoplasia, grade not otherwise specified
CIS	Squamous-cell carcinoma in situ
Conv.	Conventional (cytology screening)
GP5+/6+	Consensus primers used in PCR amplification
DNA	Deoxyribonucleic acid
EIA	Enzyme immunoassay
E6	Human papillomavirus early gene 6
E7	Human papillomavirus early gene 7
FCO	Finnish Cancer Organisations
FCR	The Finnish Cancer Registry
FDA	Food and Drug Administration
HC2	Hybrid Capture 2
HC2+	Women who (or sample which) tested positive by the Hybrid Capture 2
HDR	Care Registers for Social Welfare and Health Care (formerly known as the Finnish Hospital Discharge Register)
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HPV	Human papillomavirus
HR	Hazard ratio
hrHPV	High-risk human papillomavirus
HSIL	High-grade squamous intraepithelial lesion
IARC	International Agency for Research on Cancer
ICC	Invasive cervical cancer
ICD-10	International Classification of Diseases - 10th edition
ICD-O-3	International Classification of Diseases for Oncology -3rd edition
Ki-67	A nuclear protein that is strictly associated with a cell proliferation
L1	Human papillomavirus late gene 1

LBC	Liquid-based cytology
LLETZ	Loop electrosurgical excision procedure (also called as LEEP)
LSIL	Low-grade squamous intraepithelial lesion
LSIL+	Low-grade squamous intraepithelial lesion or worse
MAL	T-lymphocyte maturation associated protein
MGP	Modified general primer used in PCR amplification
mRNA	Messenger RNA
MSR	The Mass Screening Registry
MY09/11	Degenerate primers used in PCR amplification
N/A	Not applicable or not available
NASBA	Nucleic acid sequence-based amplification
NPV	Negative predictive value
OR	Odds ratio
p16-INK4A	Cyclin-dependent kinase inhibitor (a cell cycle regulatory protein)
PAF	Population attributable fraction
Pap	Papanicolaou
PCR	Polymerase chain reaction
PCR-	Women who (or a sample of which) tested negative by the PCR
PPV	Positive predictive value
RCT	Randomised controlled trial
Rlu	Relative light units
RNA	Ribonucleic acid
RR	Relative rate or risk
SCC	Squamous cell carcinoma
SPF10	Primers that amplify only a short sequence in PCR amplification
STM	Standard transport medium
TBS 2001	The Bethesda system, version updated in 2001
THL	Terveyden ja Hyvinvoinnin laitos (Finland´s National Institute of Health and Welfare)
UK	The United Kingdom
U.S.	The United States
WHO	World Health Organization

5 INTRODUCTION

Cervical cancer is an optimal disease for screening. It has an early asymptomatic stage which can be discovered based on the microscopy of stained exfoliated cells from the cervical epithelium. The method, Papanicolaou (Pap) smear, was invented in the 1940s.

Screening based on Pap smears attempts to identify abnormal cells that indicate the presence of a precancerous lesion. After a histological confirmation by colposcopy, lesions can be treated so that invasive disease will never develop. Screening for cervical cancer with the Pap smear has resulted in a substantial reduction in cervical cancer incidence and mortality rates in many countries. In other countries, however, the positive effects that should follow systematic screening are still yet to take root. The most likely reason for this failure is lack of resources and poor existing infrastructure for the screening programmes. Thus, cervical cancer remains an important public health problem in Europe and beyond (IARC 2005, IARC 2007, Arbyn 2009b, Bruni et al. 2010). The disease also affects rather young and fertile women, which means that the impact on the individual and on the society as a whole is greater than effects of most other cancers.

Screening with conventional cytology has been criticized as it needs functional infrastructure and rigorous quality control in order to maintain high programme sensitivity (Arbyn et al 2008a, Lönnberg et al. 2010). Furthermore, interpretation of Pap smear is subjective, and there is an increasing interest towards more objective molecular tests.

Persistent infection with a carcinogenic HPV type is a necessary, although not sufficient, cause of cervical cancer. The viral aetiology in cervical carcinogenesis has resulted in the introduction of various HPV detection assays that rely on the detection of viral nucleic acids. Lately, HPV DNA testing has emerged as a very sensitive screening test that can detect precancerous cervical lesions earlier than cytology. However, the very high sensitivity of the screening test may also have some negative implications as it may detect non-progressive cervical lesions as well.

In Finland, screening is a part of the preventive health care provision that municipalities provide for their inhabitants. In such a setting, the public health perspective is very important. When considering any new interventions, the balance between positive health outcomes and adverse side-effects of screening must be evaluated. The ideal approach is a randomised study and preferably one that takes into account the overall burden of the disease in the population within a country.

To date, many developed countries have started to vaccinate adolescent girls against HPV 16 and 18. Current vaccines have demonstrated excellent efficacy not only against HPV16/18 related CIN 3+ lesions, but they have also provided cross-protection against non-vaccine types (Lehtinen et al. 2012, Wheeler et al 2012). However, vaccines do not protect against all invasive forms of cervical cancers and, furthermore, not all women will be vaccinated. Thus, screening remains an important method for cancer prevention.

The objective of this study was to evaluate the burden of cervical HPV infections in Finland and to evaluate the use of HPV DNA test in cervical cancer screening. Screening by a primary HPV DNA test was compared to that by a conventional cytology within the population-based screening programme for cervical cancer. The study was conducted by way of a randomised trial thus meaning the results are applicable for routine use.

6 REVIEW OF THE LITERATURE

6.1 Natural history and epidemiology of human papillomavirus infection

6.1.1 High-risk human papillomavirus types

Papillomaviruses are circular double-stranded DNA viruses with close to 8000 base pairs. At present about 130 types of HPV have been identified which infect skin and mucosal epithelia at specific sites of the body. Mucosotropic HPVs can be further divided into high- and low-risk types depending on their carcinogenic potential (zur Hausen 2002, Muñoz et al. 2003, de Villiers et al. 2004, Stanley 2010). Of the HPV types infecting the mucosa of the anogenital region, 12 types have been classified as group 1 carcinogens to humans and one is probably of carcinogenic (Group 2A) type. All these 13 HPV types, and also several other possibly carcinogenic types (Group 2B), belong to the same evolutionary branch of the alpha genus in the phylogenetic tree of papillomaviruses (Figure 1) (Schiffman et al. 2011, IARC 2012). This suggests that carcinogenicity reflects viral evolution (Schiffman et al. 2005).

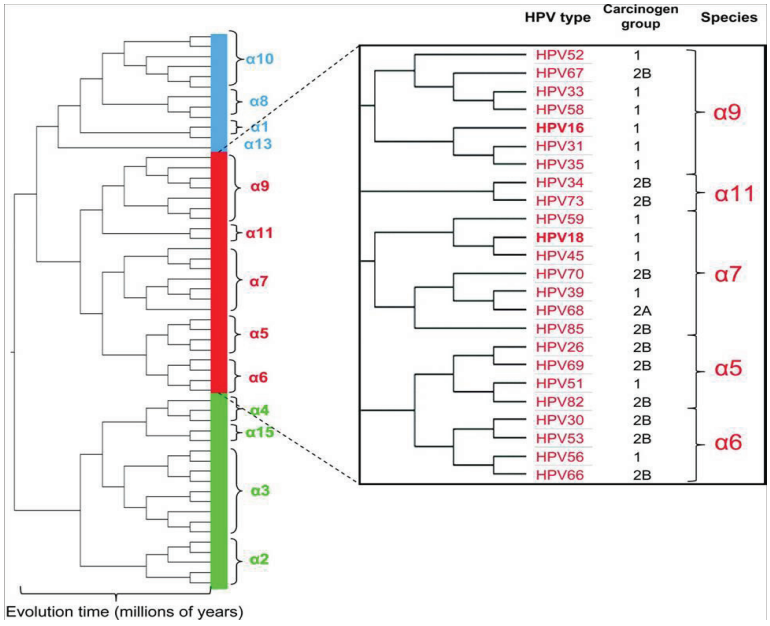


Figure 1 *Phylogenetic tree of Alpha-papillomaviruses. HPV types in red clade are associated with cervical cancer and CIN3. HPV types in blue clade cause genital warts and those in green clade cause commensal infections. Reprinted from (Schiffman et al. 2011) by permission of Oxford University Press.*

The genome organisation of HPV includes six early genes (E1, E2, E4, E5, E6 and E7) and two late genes (L1 and L2). The late genes encode coat proteins that allow genome packaging in viral capsids. The early genes encode proteins involved in viral replication and transcription as well as proteins that interfere with normal cell cycle regulation, induce genomic instability and, thus, favour cellular transformation. For reasons that are not yet clear, low-risk HPV types drive cell cycle entry in the upper epithelial layers but high-risk HPV types can stimulate the proliferation of basal cells. Obviously, high-risk and low-risk HPV types have different patterns of viral gene expression and functional differences in E6 and E7 proteins including lower affinity to pivotal proteins (Doorbar et al. 2012, Klingelhutz and Roman 2012). Between early and late sequences lies a long control region (LCR), also known as Upstream Regulatory Region (URR), which contains binding sites for both viral and cellular transcription factors (Muñoz et al. 2006, Doeberitz and Vinokurova 2009, Stanley 2010, Doorbar et al. 2012). The phylogenetic tree is based on the alignment of concatenated early and late open reading frames (Schiffman et al. 2005).

HPVs most often cause clinically relevant lesions at the uterine cervix where two types of the epithelial tissue converge. The non-keratinized stratified squamous epithelium covers the vagina and most of the ectocervix whereas glandular (mucus-secreting columnar) epithelium covers the endocervical canal and glands (crypts). The interface between the ectocervical and endocervical mucosa, a transformation zone, is a place where columnar epithelium is slowly replaced by squamous epithelium by active metaplastia. The cells in the transformation zone are especially susceptible to the HPV-mediated neoplastic transformation (Doorbar et al. 2012) and this is the area where the most squamous-cell carsinomas develop (IARC 2007). Recent findings have suggested that there are specific cells in the junctional region which have a distinct biological phenotype, i.e. a gene expression profile, from the squamous and from the glandular cells. Infection of squamocolumnar junction cells may underline the development of cervical cancer (Herfs et al. 2012).

6.1.2 HPV pathogenesis and life cycle

The life cycle of papillomavirus differs from all other virus families as the HPV needs epidermal or mucosal epithelial cells that are actively proliferating. Virus then relies primarily on the cellular machinery of these basal layer cells to replicate its DNA (zur Hausen 2002, Muñoz et al. 2006, Stanley 2010). In multi-layered stratified epithelium, the HPV is thought to access the basal lamina through minor lacerations i.e. microwounds. In the cervix the virus has a short route to these basal cells via the squamocolumnar junction.

Within the nucleus of the basal cell, the viral genome stays as an extrachromosomal episome which is expressed as a low copy number. The most genetic activity of the virus appears to be blocked but a limited expression of early viral genes increases the proliferation of the infected cells. This is consequently referred as the “non-productive” or “latent” stage of infection. The productive stage of infection begins when the daughter cells are pushed towards the epithelial surface and they start to terminally differentiate. In the suprabasal layers, an expression of the early viral genes is arrested and the late viral

genes are expressed resulting in replication of viral genome and structural proteins (Figure 2). In the most upper layers of the epithelium (mucosa or epidermis), viral particles are assembled and released within epithelial squamae (zur Hausen 2002, Muñoz et al. 2006, Doeberitz and Vinokurova 2009, Stanley 2010, Doorbar et al. 2012, IARC 2012).

Only one infected basal cell seems to acquire a disease-related phenotype that leads to expansion of the infected cell clone while other cells may carry the virus without any pathogenic effects (Doeberitz and Vinokurova 2009). The molecular events that initiate the uncontrolled (transforming) mode of HPV gene expression are not yet understood. It is known that in transforming infection deregulated expression of the viral oncogenes E6 and E7 occur in infected basal and parabasal cells. E6 and E7 have several targets of which the most characterized are the tumor suppressor proteins p53 and retinoblastoma which are associated with chromosomal instability and malignant transformation (Wentzensen et al. 2004, IARC 2005, Muñoz et al. 2006, Doeberitz and Vinokurova 2009).

In most invasive cervical cancers, hrHPV genomes are integrated into the host epithelial genome. Integrated hrHPV DNA can also be found in high-grade precancerous lesions. The integration is an important, but not an exclusive, molecular event in the cervical carcinogenesesis. The hrHPV genome integration seems to originate from chromosomal instability and occurs randomly throughout the genome. Low-risk HPV types are very rarely found integrated in tumours (Wentzensen et al. 2004, IARC 2007).

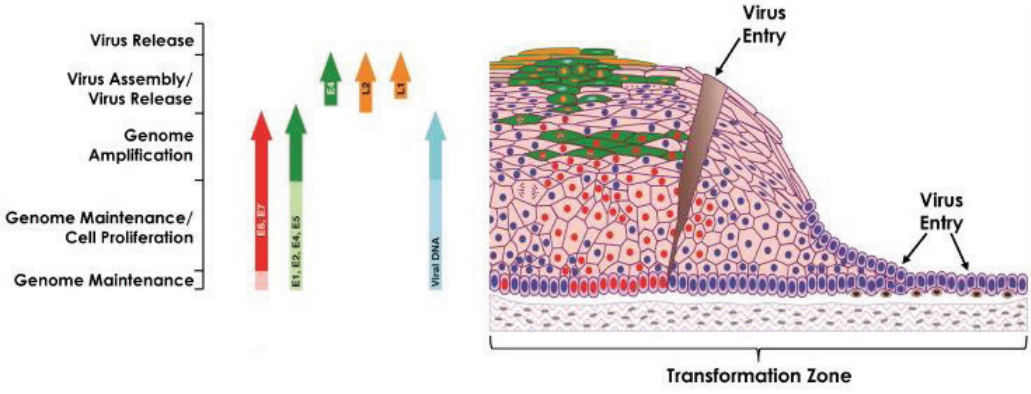


Figure 2 *HPV life cycle. Adapted from The Vaccine, 30S, Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, Stanley MA. The biology and life-cycle of human papillomaviruses, F55-F70. Copyright (2012), with permission from Elsevier.*

6.1.3 Transmission and risk factors for HPV infection

The main route of genital HPV infection is through sexual intercourse. The virus also easily transmits through skin-to-skin or skin-to-mucosa contact (Stanley 2010, IARC 2012). Thus, HPV prevalence is very high among sexually active, young people within a few years of sexual debut (Koutsky et al. 1992, Ho et al. 1998, Woodman et al. 2001,

Rodriguez et al. 2007, Stanley 2010). Also multiple HPV infections are very common among young women (Cuschieri et al. 2004a, Trottier et al. 2006, Kjaer et al. 2008).

Risk factors that are associated with cervical HPV infection include a new sexual partner and number of lifetime sexual partners both in women and men (Deacon et al. 2000, de Sanjose et al. 2003, Herrero et al. 2005, Castellsague et al. 2006, Muñoz et al. 2006, Vaccarella et al. 2006a, Stamataki et al. 2010, Camargo et al. 2011, Miranda et al. 2012). Age at first intercourse does not seem to be an independent risk factor for HPV infection (Deacon et al. 2000, Herrero et al. 2005, Vaccarella et al. 2006b, Camargo et al. 2011). It does, however, predict the presence of cervical intraepithelial lesion grade 3 (CIN 3) in the same way as a long time since a new sexual partner among HPV infected women. These results are derived from a nested case-control study within a cohort in Manchester, UK. The study explained results so that those women who were in long-term relationships, and who remained infected throughout, were carriers of a persistent infection (Deacon et al. 2000).

HPV infection has been shown to be more common and more likely to persist in women with human immunodeficiency virus (HIV). This appears to be related to alterations in cell-mediated immunity, increased susceptibility and possibly due to the re-activation of latent HPV infection in HIV infected persons (Denny et al. 2012).

In addition to direct risk factors, there are sociodemographic factors such as age, geographic region and marital status that are associated with HPV infection (Camargo et al. 2011, de Sanjose et al. 2003, Herrero et al. 2005, Ronco et al. 2005, Kahn et al. 2007, Stamataki et al. 2010). These factors likely reflect the sexually transmitted aetiology of the infection and many of them are confounded by each other, especially by age. Furthermore, a low socioeconomic status (SES), measured through factors such as income, education, occupation and living conditions, has been established a risk factor for cervical cancer. This has been explained by differences in access to and compliance with cervical screening, but also by differences in sexual behaviour, and therefore the effect of the HPV infection (de Sanjose et al. 1996, Parikh et al. 2003, Khan et al. 2005a, Kahn et al. 2007, Stamataki et al. 2010, Drolet et al. 2013).

6.1.4 Persistence and clearance

Most HPV infections are transient and they are cleared within months. How the infection manages to escape the immune system even for so long is an important question. Due to a restricted replication mode, HPVs avoid major tissue damage and inflammation which delays the contact between viral antigens and antigen-presenting cells of the host immune system (Doeberitz and Vinokurova 2009). The median time for viral clearance is approximately 8 months (Ho et al. 1998, Dalstein et al. 2003). Non-oncongenic HPV infections are generally cleared in 4 to 9 months whereas hrHPV infections persist from 12 to 18 months (Stanley 2010). Up to 90% of all HPV infections regress spontaneously in 2 years (Ho et al. 1998, Richardson et al. 2003, Moscicki et al. 2004, IARC 2007, Rodriguez et al. 2007). This viral clearance is due to an effective cell-mediated immunity accompanied usually, but not necessarily, with a seroconversion and antibody production

towards the major coat protein L1 (Stanley 2010). A failure to clear or control infection results in a persistence which in turn increases the probability of progression to high-grade cervical lesion and invasive carcinoma when the persistent infection is caused by a hrHPV type (Ho et al. 1998, Stanley 2010, Doorbar et al. 2012).

It is currently believed that HPV clearance occurs when viral gene expression is shut-off by immune response accompanied with infiltration of predominantly T-lymphocytes. A recent study using a laser capture approach demonstrated that a viral genome persisted in the epithelial basal cells even when the virus was not detectable using standard in situ hybridisation methods. This suggests that viral genome may reside as a latent infection in a small subset of basal cells after immune regression. Reactivation of latency is prevented by host immune surveillance but it may recur following a change in immune status (Maglennon et al. 2011, Doorbar et al. 2012).

HPV 16 is likely to persist longer than other hrHPV types and, in the process, is likely to cause neoplastic progression (Richardson et al. 2003, Schiffman et al. 2005). For some HPV types, particularly for HPV 16, variant lineages representing further evolutionary divergence also differ in their risk of viral persistence and risk of cancer (Schiffman et al. 2010). Infection with multiple HPV types has been associated with lower rate of HPV clearance (Ho et al. 1998, Moscicki et al. 2004, Louvanto et al. 2010). New infections with hrHPV types are associated with low absolute risk of HPV persistence. The risk of persistence has increased with the increasing age of the women in some studies (Ho et al. 1998, Rodriguez et al. 2010) but this association has not been confirmed in all studies (Dalstein et al. 2003, Maucort-Boulch et al. 2009, Louvanto et al. 2010).

6.1.5 Natural history of cervical intraepithelial neoplasia

High-risk HPV infection is the major risk factor for the development of both squamous-cell and adenocarcinoma of the cervix (Stanley 2010). However, the natural history of squamous-cell carcinoma through precancerous stages is better known than that of AIS and adenocarcinoma (Krivak et al. 2001). The development of cervical cancer is a multi-step process in which only the last step leading to an invasive lesion is not reversible (Figure 3). Otherwise precancerous lesions persist, progress and regress over time depending on the characteristics of both woman and the precancerous lesion. Thus, estimated rates of progression have varied significantly between studies. Generally, CIN 1 lesions are only a manifestation of viral infection. They can be caused by low-risk HPV types alone and their risk of progression is low. High-grade cervical lesions, i.e. CIN 2 and 3 are predominately associated with hrHPV types. They are more persistent and their risk of progression is higher.

In terms of CIN lesions of any grade, up to 90% regress spontaneously in women aged 13 to 22 years (Moscicki et al. 2004) whereas among women 34 years and older, the estimated rate of regression is 40% (van Oortmarssen and Habbema 1991). In one study, 77% of the most severe preinvasive lesions, carcinoma *in situ*, regressed spontaneously among women younger than 40 years-old whereas 61% among women aged 40 and older (Boyes et al. 1982).

A tendency of spontaneous regression decreases with aging of women and with increasing CIN grade. In a prospective Finnish study, 528 women with mild cervical lesions were followed for a period of 6 years. Of CIN 1 lesions, 55.7% were estimated regressive and 14.2% progressive. The corresponding estimates of regression and progression for CIN 2 lesions were 53% and 21% and for CIN 3 lesions 14% and 69%, respectively (Syrjänen et al. 1992).

Hakama and Räsänen-Virtanen (1976) published a modelled estimation based on Finnish Cancer Registry data which suggested that among women targeted by the screening programme (from 30 to 60 years-olds) 28-39% of severe dysplasia and carcinoma *in situ* lesions would progress to invasive cancer (Hakama and Räsänen-Virtanen 1976). This is close to the estimated percentage of 36% as presented in a literature review (Mitchell et al. 1996). A study from New Zealand reported similar progression rates as 20% of women with untreated CIN 3 lesions developed a cancer of cervix or vaginal vault within 10 years and 31% within 30 years (McCredie et al. 2008).

The development of cervical cancer is a slow process in which the median time from the initial exposure to HPV to the development of the carcinoma *in situ* is at least 7 to 12 years (Ylitalo et al. 2000). In a model-based study of a screening programme in the British Columbia, Canada, the average duration of the dysplasia and CIS stages combined was estimated at 11.8 years (van Oortmarssen and Habbema 1991). Considering cervical cancer prevention, the tiny lesions found with repetitive screening might have higher regressive potential in addition to longer latency before cancer development (Rodriguez et al. 2007).

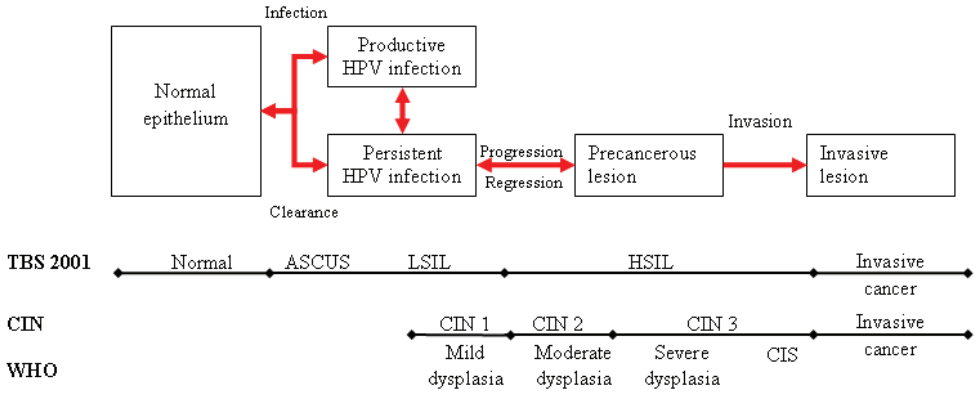


Figure 3 *Progression model of squamous-cell carcinoma. Correlation between cytology and histological diagnosis is only suggestive. ASC-H does not have a clear position on the line.*

6.1.6 Cofactors of HPV in progression to precancer and cancer

Persistent hrHPV infection is a necessary cause of cervical cancer (Walboomers et al. 1999, Bosch et al. 2002). As only a small fraction of infections progress into high-grade cervical lesion and cancer, there must be additional viral, host or environmental factors contributing to the viral persistence and progression. However, the role of cofactors is difficult to study as many of them correlates with sexual behaviour (IARC 2012).

One of the most studied cofactors is smoking with approximately 2-fold risk. Smoking, unlike other cofactors, seems to be a risk factor only for SCC but not for adenocarcinoma (Berrington de Gonzalez et al. 2004, Castellsague et al. 2006, Muñoz et al. 2006, IARC 2012). Smoking is believed to influence the natural history of cervical cancer through direct effect of carcinogenic constituents of cigarette smoke and through alterations in the immune system. It has also been suggested that smoking status correlates with screening participation which would explain the increased risk for cervical cancer among smokers. A prospective Danish study in which hrHPV-positive women were followed for up to 13 years found increased risk for CIN 3+ associated with both long-term (≥10 years) and heavy-smoking (≥ 20 cigarettes/day). The number of annual Pap-smears did not explain the differences in risk by smoking status (Jensen et al. 2012).

Another established risk factors include the number of full time pregnancies (Muñoz et al. 2002, Castellsague et al. 2006, IARC 2012) and the immunosuppression in transplant recipients and in HIV-infected subjects (IARC 2007, Doeberitz and Vinokurova 2009, Denny et al. 2012). Long-term use of oral hormonal contraception has also been associated with an increased risk of cervical cancer and its precursors (Muñoz et al. 2006, IARC 2012) but it has not been evident in all studies (Deacon et al. 2000). Furthermore, among women with a persistent hrHPV infection, any use of oral contraceptives was associated with a decreased risk for CIN 3+ in the prospective setting (Jensen et al. 2013). It is difficult to establish whether oral contraceptive use is an independent risk factor or only reflects sexual habits. Use of an intrauterine device (IUD) is not associated with CIN or cancer development and it may even reduce the risk of adenocarcinoma (Castellsague et al. 2006, Jensen et al. 2013).

Other sexually transmitted infections as well as general inflammation reaction associated with various infectious agents have been associated with the risk of progression to precancer or cancer (IARC 2012). Even though not evident in all studies (Castellsague et al. 2006), the most abundant and consistent evidence exist for Clamydia trachomatis (Muñoz et al. 2006, IARC 2012). However, the correlation is so high between the different sexually transmitted agents that it is difficult to rule out confounding with HPV (IARC 2012).

Cervical cancer patients have shown reduced or non-existent immunological responses. This suggests that HPV persistence may be associated with a failure to develop an immune response or an inability to recognise viral antigens (Doorbar et al. 2012). Genetic variability of the host, especially the genes controlling the immune response such as human leukocyte antigen (HLA) are important determinants of HPV persistence and disease progression (de Auraujo Souza et al. 2009).

6.2. Burden of human papillomavirus and related diseases

6.2.1 Type-specific HPV prevalence

Meta-analyses or systematic reviews on the prevalence of HPV DNA are usually restricted to women with normal cytology. These allow for better comparability between studies (de Sanjose et al. 2007, IARC 2007, Bruni et al. 2010). The most comprehensive study on HPV types among women with normal cytology comprised over 1 million women and 194 studies of which the vast majority (76.3%) was derived from routine cervical screening programmes. The five most common HPV types worldwide were HPV16, 18, 52, 31 and 58 (Bruni et al. 2010), which together contributed to 50% of all HPV infections (de Sanjose et al. 2007). HPV 16 alone contributed to 22.5% of the global HPV infection burden. However, it must be noted that this contribution of HPV 16 was inversely correlated (correlation coefficient -64.8%; P=.017) with the overall HPV prevalence in the population (Bruni et al. 2010).

Regarding cervical cancer screening, it is important to find type-specific prevalence of hrHPV types in representative samples of screening population in each country. This data is needed also to monitor the impact of the HPV vaccination. The characteristics of the population-based studies on type-specific HPV prevalence among women attending cervical cancer screening in Europe are summarised in Table 1. If several studies were available from the same country, the best available and the most recent data favouring screening target age groups was chosen. Large screening trials and organised cervical screening programmes, in which women are actively invited for screening, were the preferred evaluation methods for detecting the presence of type-specific infection in the population.

Representative type-specific reports from Eastern Europe are limited. In addition to the three studies cited here (Bardin et al. 2008, Shipitsyna et al. 2011, Ucakar et al. 2012), high-risk HPV type results from Belarus, Latvia and Russia have been reported in studies from the NIS cohort of 3 187 women. In that cohort half of the participants were attending routine screening whereas other women were either gynaecologic outpatients or attending sexually transmitted disease clinics. Therefore, that data is not further considered here (Kulmala et al. 2007). Nor was a study from Norway included as it only covered a subset of hrHPV types (Molden et al. 2005).

The prevalence of hrHPV infection is highly variable in Europe. At 22.8%, it was exceptionally high in Denmark. Elsewhere it varied from 2.4% (Spain) to 16.1% (France). HPV 16 was the most common type in all countries, with the average prevalence of 3.1% and a range from 0.9% (Spain) to 6.0% (Denmark). HPV 16 was followed by HPV 31 in the majority of the countries, with the average prevalence of 2.0% in nine studies from all subregions of Europe. However, it shared the second place with HPV 52 in Germany and Denmark and with HPV 35 in Greece. The second most frequent types were HPV 18 and HPV 51 in Scotland, HPV 45 in Italy, HPV 51 in France and HPV 56 in Poland. HPV 18 was among the three most often detected types in all three studies from the UK and in the study from the Netherlands (Table 1).

Table 1. *Overall and type-specific prevalence of hrHPV types by PCR amplification among women attending cervical cancer screening in Europe*

Country Reference	Age range	No	Primers	hrHPV (%)	Specific Group 1 HPV type (%)											
					16	18	31	33	35	39	45	51	52	56	58	59
Greece Agorastos et al. 2009	20-59	4 139*	MY09/11	5.9†	1.4	0.3	0.6	0.1	0.6	0.1	0.1	0.4	0.2	0.1	0.2	0.1
Belgium Arbyn et al. 2009a	14-97	9 284	Other§	15.2	3.7	1.5	3.0	0.8	0.5	1.5	0.5	2.3	1.6	1.1	1.1	1.7
Poland Bardin et al. 2008	18-59	834‡	GP5+/6+	11.3	3.7	0.7	1.4	1.1	0.4	0.4	1.6	1.1	1.4	1.7	0.8	0.4
Netherlands Coupé et al. 2008	18-65	45 362	GP5+/6+	5.6	1.8	0.5	0.8	0.4	0.2	0.3	0.4	0.4	0.4	0.4	0.3	0.1
UK (Scotland) Cuschieri et al. 2004a	16-78	3 444	GP5+/6+	15.7	6.4	2.2	2.1	1.2	0.0	1.1	1.4	2.2	1.8	1.3	1.1	1.3
Spain de Sanjose et al. 2003	14-75	973‡	GP5+/6+	2.4	0.9	0.0	0.4	0.0	0.5	0.1	0.0	0.4	0.1	0.1	0.1	0.2
Sweden Forslund et al. 2002	32-38	6 123	GP5+/6+	6.8	2.1	0.6	1.1	0.4	0.3	0.2	0.8	0.4	0.3	0.5	0.3	0.1
UK (South Wales) Hibbitts et al. 2008	20-65	9 079	GP5+/6+	11.1	3.5	2.4	2.5	1.7	1.2	1.4	1.5	1.1	0.9	1.2	2.2	1.2
Denmark Kjaer et al. 2008	15-93	11 600	SPF-10	22.8†	6.0	2.7	4.5	2.1	1.0	2.6	2.1	4.4	4.5	2.0	1.3	1.2
Germany Klug et al. 2007	≥ 30	8 101*	PGMY09/11	4.3	1.3	0.4	0.5	0.2	0.1	0.4	0.4	0.4	0.5	0.1	0.3	0.2
France Monsonego et al. 2012	25-65	4 487	Other§	15.1†	2.3	0.4	1.6	0.3	0.2	0.9	0.4	2.1	0.5	1.7	0.4	0.5
Italy Ronco et al. 2005	25-70	1 013	GP5+/6+	7.1	2.6	0.1	0.5	0.2	0.1	0.3	0.6	0.2	0.2	0.2	0.3	0.0
UK (Manchester) Sargent et al. 2008	20-64	24 470	PGMY09/11	10.6	3.3	1.3	1.3	0.7	0.4	1.1	0.8	1.2	1.5	0.7	0.7	0.8
Russia Shipitsyna et al. 2011	30-65	823	Other§	13.0	3.9	0.5	2.8	1.3	0.4	0.4	0.7	0.6	1.7	0.9	0.5	0.4
Slovenia Ucakar et al. 2012	20-64	4 431	PGMY09/11	12.9	3.5	1.0	2.6	0.7	0.2	1.1	0.9	1.8	1.8	0.7	0.6	1.1

* Opportunistic screening

† Overall hrHPV prevalence from HC2-positives; LR probes were also used in the study by Kjaer et al. 2008

‡ Population-based sample

§ Taqman real-time PCR targeting type-specific sequences of viral E6 and E7 genes in Arbyn et al. 2009a; PapilloCheck assay using primers that target E1 region of the HPV genome in Monsonego et al. 2012

In Europe, a noticeable North to South gradient exists. This means that HPV prevalence decreases with decreasing latitude (Bruni et al. 2010). However, reports from individual countries do not necessarily follow strictly to geographical subregions. For instance, rather low prevalence rates were seen in the most Northern country (Sweden) whereas somewhat high prevalence rates were observed in Denmark, France, and Belgium. There are also remarkable differences in the prevalence estimates not only between countries but also among studies within the same region (Bruni et al. 2010). This may be due to different settings and screening methods but also due to the differences in the self-selection of women to attend screening (IARC 2007, Thulaseedharan et al. 2013).

The numbers of HPV types detected or classified as high-risk in individual studies have an effect on overall hrHPV prevalences. The categorization of hrHPV types was most stringent in the studies from Germany, Russia and France in which 13 types were considered as high-risk. In contrast, the study from Scotland classified up to 18 types as high-risk for cervical cancer (Table 1).

In Belgium, France and Poland, all samples were HPV typed but in most studies referred here only hrHPV DNA positive samples were subjected to genotyping. When genotyping followed HC2-positive samples, a cutoff of 1.0 relative light units (RLU/Co ratio) was generally used. In the Slovenian study, all specimens were first tested with HC2 and RealTime High Risk HPV Test (Abbott, Wiesbaden, Germany). All samples with concordant or discordant positive results were then genotyped (Ucakar et al. 2012). In two studies HC2-negative samples were genotyped as controls. 50 randomly selected HC2-negative samples were all negative by genotyping in Greece (Agorastos et al. 2009) whereas in the German study, 21 out of the 191 HC2-negative samples included known HPV types and in four samples HPV DNA was detected, but the type could not be defined (Klug et al. 2007).

The age-distribution of the population being studied always warrants a careful attention as the prevalence of HPV is strongly age-related (Petignat et al. 2005, IARC 2007, Klug et al. 2007, Bardin et al. 2008, Coupe et al. 2008, Kjaer et al. 2008, Nielsen et al. 2008, Agorastos et al. 2009, Arbyn et al. 2009a, Shipitsyna et al. 2011, Ucakar et al. 2012). The highest overall hrHPV prevalence seen in the Danish study is partially explained by the age of women screened. The mean age of women was 36.4 years, one of the lowest among studies presented here, and the hrHPV prevalence was extremely high 44.7% (95% CI 42.7-46.7) in women from 20 to 24 years of age (Kjaer et al. 2008).

Furthermore, HPV prevalence has been shown to vary according to the HPV testing method used. The SPF10 primer used in the Danish study amplifies only a short sequence in PCR amplification (Arbyn et al. 2008a). It has been accompanied with the highest HPV detection rates and MY09/11 consensus primers result in higher HPV prevalence rates than GP5+/6+ primers (Bruni et al. 2010). On the other hand, a previous study on HPV prevalence in Denmark performed in the early 1990s with a similar HPV typing method showed a substantially lower overall high-risk prevalence, i.e. 17.9% among women aged 20–29 years and 4.4% among women aged 40–50 years (Nielsen et al. 2008). Two plausible explanations would be a cohort effect and the fact that previous study tested cervical swaps using only the HR probes of the HC2 test kit whereas the latter study used LBC samples and both HR and LR probes.

6.2.2 HPV types and cervical cancer

There are very high rates of incidence of and mortality from cervical cancer in Eastern Europe. This has been associated with increased exposure to HPV infection and to a lack of effective screening programmes (Arbyn et al. 2009b, Arbyn et al. 2011, Shipitsyna et al. 2011). The observed rates of hrHPV types from Russia were lower than those reported from Belgium even though the genotyping method and age of participants were similar. Moreover, Russian women had likely an increased risk for cervical HPV infection as about 15% of them had symptoms of a urogenital infection (Shipitsyna et al. 2011).

HPV 16 and HPV 18 remain underrepresented in women with normal cytology but they persist and progress more often than other hrHPV types (Khan et al. 2005b, Kjaer et al. 2010) which explain their importance in high-grade cervical lesions. Consequently, HPV types 16, 18 and 45 are significantly over-represented in ICC compared to high-grade cervical lesions ICC (Smith et al. 2007).

A study of 10 575 cases of invasive cervical cancer (ICC) (de Sanjose et al. 2010) and a meta-analysis of more than 30 000 ICC cases (Li et al. 2011) have confirmed that HPV types 16 and 18 account for more than 70 % of ICC cases worldwide. Together with these, the next most common types of HPV (31, 33, 35, 45, 52 and 58) account for up to 90% of the global cervical cancer burden. HPV 16 and HPV 18 are the most common types found in ICC everywhere and the proportion of cases associated with HPV16/18 appears similar across all regions. Also the following six types show only minor variation in their relative importance by geographical region (de Sanjose et al. 2010, Li et al. 2011).

The five most commonly found types among European women with ICC are 16, 18, 31, 33 and 45 whereas those with a high-grade cervical lesion (including both cytologically diagnosed HSIL and histologically diagnosed CIN 2, CIN 3 or carcinoma *in situ* lesions) are 16, 31, 33, 18, and 58 (Smith et al. 2007, Li et al. 2011). HPV 16 and HPV 18 contributed to 52% and 6% of HSIL lesions in Europe, respectively (Smith et al. 2007). Studies listed in Table 1 using the year 2001 version of the Bethesda System (TBS) (Solomon et al. 2002), showed that the prevalence of HPV 16 infection among women with HSIL cytology was lowest in Denmark and Belgium (35%) and highest in Slovenia (50%). HPV 18 contributed to 8% of all HSIL lesions in Slovenia, 15% of those in Denmark and none in Belgium and Russia. The prevalence of HPV 18 was exceptionally high (33%) among women with severe dysplastic cells in Germany using different cytological classification (Petry et al. 2003, Klug et al. 2007).

Generally only a few cases of invasive cancer were detected in each study. Poland was an exception with 88 cases of ICC, of which 84 were squamous cell carcinomas and four adenocarsinomas. HPV 16 was prevalent in 73.9% of cases of ICC and HPV 18 in 5.7%. Women with ICC showed a prevalence ratio of 3.9 (95% CI 2.4-6.2) if infected with HPV 16 and 1.3 (95% CI 0.8-2.5) if infected with HPV 18 compared to women with normal cytology (Bardin et al. 2008).

6.2.3 Age-specific HPV prevalence

The overall HPV prevalence has been estimated to be 11.7% worldwide. With Sub-Saharan Africa and Eastern Europe showing the highest rates, it is clear that the prevalence rate varies greatly by geographical regions (Bruni et al. 2010). HPV infections and their clearance are very common at young ages. This is reflected as a sharp peak in the HPV prevalence curve following population norms of sexual initiation (Koutsky et al. 1992, Ho et al. 1998, Woodman et al. 2001, IARC 2007, Rodriguez et al. 2007, Stanley 2010). HPV prevalence then declines rather constantly over ages. In some regions, a second increase in the prevalence has been observed during midlife (Figure 4). This is lower for non-carcinogenic than for hrHPV types (Herrero et al. 2005). There are also areas such as India where HPV prevalence never decreases markedly (Bruni et al. 2010).

It has been hypothesised that an impaired immune response as a result of hormonal changes at menopause induces reactivation of an existing but undetectable HPV infection. In addition, changes in the sexual behaviour of middle-aged women and their spouses along with cohort effects have been proposed as possible cause of the HPV infection (de Sanjose et al. 2007). With effective screening programmes for women aged up to 40 in place, the second increase in prevalence is clearly missing in the regions of Europe and Northern America (Cuzick et al. 2006, Bruni et al. 2010). Screening may not only reduce persistent HPV infections but the treatment of precancerous lesions and associated inflammatory responses may function as an immunological factor initiating anti-HPV responses (Passmore et al. 2007). Thus, the increase in prevalence at older ages might result from the interplay of individual and viral characteristics and also from screening history (Bruni et al. 2010).

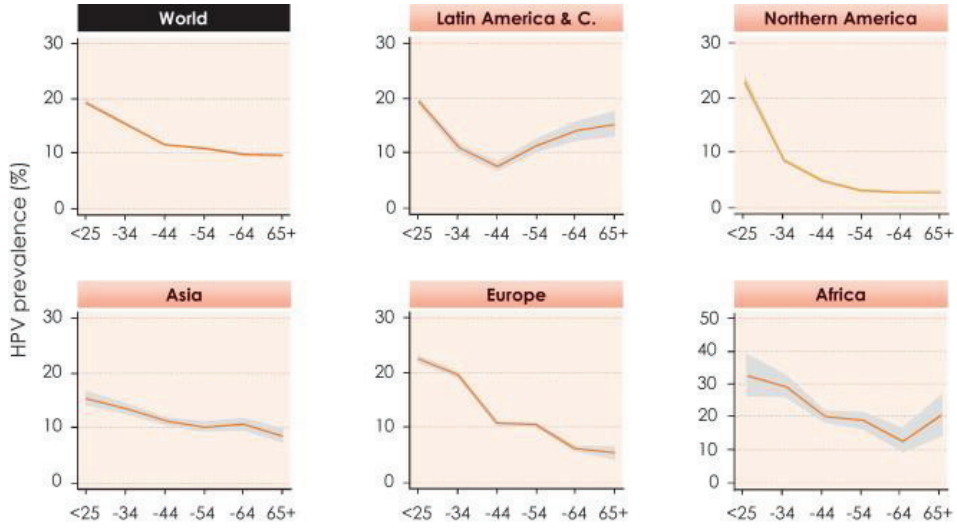


Figure 4 *HPV prevalence and 95% confidence intervals (shaded areas) by age group among women with normal cytology. C: Caribbean. Reprinted from (Forman, de Martel et al. 2012) with permission from Elsevier. Redrawn from (Bruni et al. 2010) by permission of Oxford University Press.*

Figure 5 illustrates the age-related prevalence of hrHPV infection among women attending cervical screening derived from European studies that provided data in 5-year age groups. The characteristics of the seven studies on hrHPV prevalence (Cuschieri et al. 2004a, Bardin et al. 2008, Coupe et al. 2008, Hibbitts al. 2008, Kjaer et al. 2008, Arbyn et al. 2009a, Ucakar et al. 2012) are summarised in Table 1. Three of the studies, namely those from Scotland, the Netherlands and Poland, provided age-specific data conveniently in 10-year age groups. These studies are included in Figure 5 which assumes a constant decline in prevalence and, thus, uses the average prevalence between the 10-year age groups.

In addition, three more studies were found eligible. A study from Switzerland included 7 254 women who were routinely screened by LBC and HC2 (Petignat et al. 2005). The HART study was a multicentre screening study of 11 085 women aged 30-60 years conducted in five different areas around the UK. Women with borderline cytology and those positive for hrHPV DNA using a Hybric Capture 2 assay (HC2, Digene Corp. Gaithersburg, Maryland, USA) and with negative cytology were randomised either to immediate colposcopy or to repeat testing at 12 months. Baseline data provided age distribution of positive HPV DNA test results (Cuzick et al. 2003). Age-related high-risk HPV prevalence was also available from a controlled randomised trial, the NTCC, conducted in nine organised cervical screening programmes in Italy (Ronco et al. 2008). Characteristics from the recruitment to the second study phase are given in Table 2.

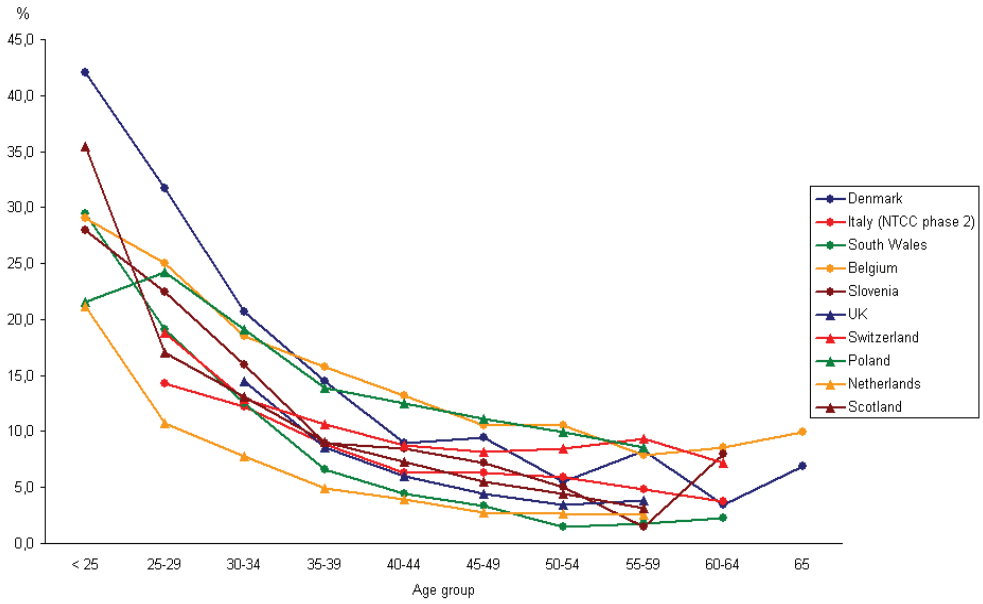


Figure 5 *Age-specific prevalence of cervical hrHPV infection in women attending cervical screening in Europe*

Table 2. *Designs and protocols of studies on HPV DNA testing in a population-based cervical cancer screening in Europe and North America*

		Control arm			Intervention arm				
Trial (country)	Age (y)	Eligible women	Screening test(s)	Indicative for colposcopy	Eligible women	Screening test(s)	Indicative for Colposcopy	Indicative for Repeat test(s)	References
ARTISTIC (UK)	20-64	6124	Co-testing LBC and HC2	moderate+ dysplasia; mild dysplasia at 6months	18386	Co-testing HC2 and LBC	moderate+ dysplasia	colpo at 6mo if LBC borderline and HPV+; colpo at 12mo if LBC abnormal or HPV+; colpo at 24mo if HPV+	Kitchener et al. 2006 Kitchener et al. 2009 Kitchener et al. 2011
ATHENA* (U.S.)	≥ 21	46887	Co-testing LBC and cobas HPV	LSIL+ HPV+ and ASCUS	46877	Co-testing cobas HPV and LBC	HPV+ and ASCUS HPV16/18+	both tests at 12 months; colpo if HPV+ and ASCUS+ or HPV16/18+	Castle et al. 2011 Wright et al. 2012
POBASCAM (Netherlands)	30-60	22106	Pap test	moderate+ dysplasia	21996	Co-testing HC2 and Pap test	moderate+ dysplasia	colpo at 6 months if still HPV+ and BMD; colpo at 18mo if HPV+	Bulkmans et al. 2004 Rijkaart et al. 2012a
CCCaST (Canada)	30-69	5059	Co-testing Pap test and HC2	ASCUS+ or HPV+	5095	Co-testing HC2 and Pap test	HPV+ or ASCUS+	N/A	Mayrand et al.2006 Mayrand et al. 2007

* Not a randomised clinical trial as all recruited women were screened by co-testing
† Number of women aged 25-34 years from the total study population in brackets
‡ Two out of nine centers referred for colposcopy based on LSIL+ whereas ASCUS indicated a repeat test

Table 2. *Continued*

		Control arm			Intervention arm				
Trial (country)	Age (y)	Eligible women	Screening test(s)	Indicative for colposcopy	Eligible women	Screening test(s)	Indicative for Colposcopy	Indicative for Repeat test(s)	References
HPV FOCAL (Canada)	25-65	6154	LBC (reflex HC2)	ASC-H, LSIL+	12494	HC2 (reflex LBC)	HPV+ and ASCUS+	both tests at 6 and 12months; colpo if ASCUS+ or two times HPV+	Ogilvie et al. 2010 Ogilvie et al. 2012
India	30-59	32058	Pap test	ASCUS+	34126	HC2	HPV+	N/A	Sankaranarayanan et al. 2009
NTCC (Italy) Phase 1	25-60	22466 (5808)†	Pap test	ASCUS+	22708 (6002)†	Co-testing HC2 and LBC	ASCUS+ or HPV+ aged 35≥ y	HPV+ aged <35y at 12 months; colpo if HPV+/ASCUS+	Ronco et al. 2006a Ronco et al. 2006b
NTCC (Italy) Phase 2‡	25-60	24535 (6788)†	Pap test	ASCUS+	24661 (6937)†	HC2	HPV+	N/A	Ronco et al. 2008 Ronco et al. 2010
Swedescreen (Sweden)	32-38	6270	Pap test	ASCUS+	6257	Co-testing GP5+/6+ and Pap test	ASCUS+	after 12 months; colpo if still HPV+ for the same type	Naucler et al. 2007b Naucler et al. 2009

Figure 5 shows that the prevalence of hrHPV infection in Europe followed a descending curve but the rates varied between countries. The highest prevalence rates were seen among the youngest women screened in all except for one study. In Poland, the prevalence was slightly lower among women younger than 25 years and the peak of 24% was seen among 25 to 29-year-olds. Among 30-year-olds women, the most frequent hrHPV rate of approximately 21% was observed in Denmark and the less frequent rate of approximately 8% was observed in the Netherlands. The lowest rates of less than 2% were seen in the study from South Wales among women from the age of 50 onwards. Following the highest value among the youngest women, rather constant decline in prevalence was seen over ages in most of the studies. There was some fluctuation in prevalence of hrHPV types in older ages but no clear second increase existed. As for cervical cancer burden in Eastern Europe, the overall prevalence of hrHPV infection and age distribution was not remarkably different in Slovenia in comparison to other European countries.

The data from the recruitment of the ARTISTIC trial in the UK provided age-specific data in 10-year age groups starting from the age of 20 and, thus, could not be included in Figure 5. However, the study provided type-specific HPV infections that had been stratified by age group. Most HPV types showed relatively minor differences in the type distribution by age. Moreover, the proportion of HPV 16 and HPV 33 infections was higher in hrHPV-positive women aged 20–29 years than among women aged 30 years and older (Sargent et al. 2008). Also the study by Coupé et al. demonstrated a significant decrease in the prevalence of HPV 16 with increasing age (OR=0.76, 95%CI 0.67-0.85) among hrHPV-positive women and a marginal decrease also for HPV types 39 and 52 (Coupe et al. 2008).

6.2.4 Other cancers attributable to HPV infection

An evaluation of the infectious agents in human cancers by the International Agency for Research on Cancer (IARC) established that HPV is a definite human carcinogen. This applies not only to cervical cancer but also to cancers other than cervix such as vulva, vagina, anus, penis and oropharynx (including base of tongue and tonsils) (Forman et al. 2012, IARC 2012, Arbyn et al. 2012a). Over 600 000 of new annual cancer cases can be attributed to HPV infection (4.8% of the global cancer burden). The majority of these are cervical cancers (86.9%) in which the population attributable fraction (PAF) of HPV infection has been estimated to be 100% (Forman et al. 2012, Arbyn et al. 2012a).

The incidence of cervical cancer has decreased over recent decades globally. At the same time the incidence of anal and oropharyngeal carcinoma, for which there are no screening programmes available, has been rising (Arbyn et al. 2012a). In a review of HPV-related cancers, PAFs varied according to the other anatomic sites, the highest being anus (88%) and following vagina (70%), penis (50%) and vulva (43%). For oropharyngeal cancer the overall estimate was 25.6% but with large geographical variability with a maximum estimate of 56% in North America (de Martel et al. 2012). The contribution of HPV to cancer burden is highly dependent upon the level of institutional development of the particular region (Forman et al. 2012).

6.3 Screening for cervical cancer

6.3.1 Benefits of cytology screening

The Wilson and Junger criteria emphasise the important features of any screening programme. The disease has to be an important health problem. The natural history of the disease has to be understood and there should be a recognisable latent or early symptomatic stage. There has to be a suitable test which is accepted by the population. There should be an agreed policy on whom to treat as patients and exist an accepted treatment for the disease. Screening is a continuous process and diagnosis and treatment in the programme should be cost-effective (Wilson and Jungner 1968).

Knowing the natural history of HPV infection and CIN underlies the planning of preventive efforts for cervical cancer screening as a public health activity. The long time required for the development of ICC enables to catch the cancer at the symptomatic preclinical phase or already as a precancerous lesion so that ICC will never develop. The peak of CIN 3 lesions follow the peak on HPV prevalence 5 to 15 years later, depending on the intensity of the screening (Schiffman et al. 2011). Furthermore, those women who are diagnosed with screen-detected invasive cancer tend to be more than 10 years older than those women who are diagnosed with CIN 3 (IARC 2007). The cervical disease burden has been estimated by the National Expert Group on HPV Related Disease Prevention (Figure 6).

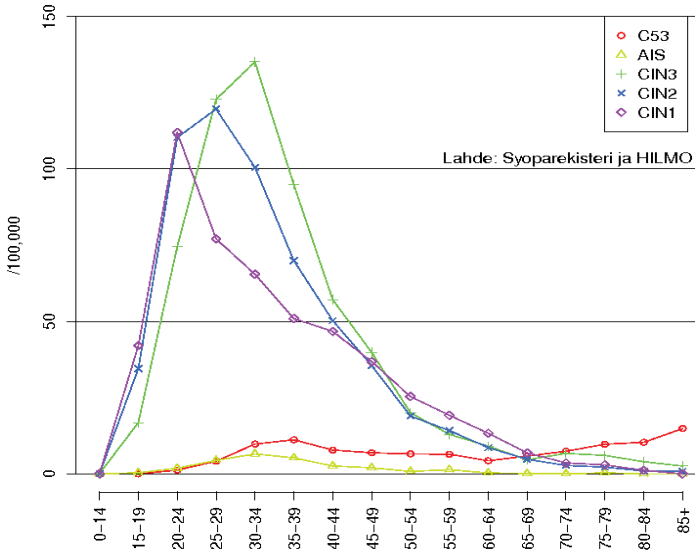


Figure 6 *The incidence of cervical precancerous lesions and cancer in Finland in 2004–2008 by age group. C53= ICC. Reprinted from National Expert Group on HPV related Disease Prevention. Report 28/2011. The National Institute for Health and Welfare: Helsinki.*

The ultimate goal of cervical cancer screening is to reduce mortality from cancer of the uterine cervix. Furthermore, the detection and treatment of precancerous lesions can also decrease the incidence of cervical cancer. The efficacy of conventional cytology screening has never been demonstrated in randomised clinical trials. Still, it has been estimated that more than 80% of cervical cancers and cervical cancer deaths could be prevented in an organised setting through regular screening and with appropriate quality control measures in place at all levels (Hakama and Räsänen-Virtanen 1976, Anttila et al. 1999, IARC 2005, Arbyn et al. 2008a). Screening with cytology has been shown to be less effective in preventing adenocarcinomas and deaths from it than invasive squamous cell carcinomas (Nieminen et al. 1995, Sasieni et al. 2009, Lönnberg et al. 2012).

6.3.2 Accuracy of cytology

Screening with cytology is based on the microscopy of stained exfoliated cells from the cervical epithelium. Interpretation of the Pap smear is based on a subjective assessment by cytotechnicians and cytopathologists who try to identify abnormal cells and cell clusters that indicate the presence of a precancerous lesion or cancer.

The accuracy of the conventional cytology (Pap test) is highly variable and it is affected by the age and screening history of the women. Also the study design and issues of selection in attendance and diagnostic confirmation of the disease have an effect on it (Arbyn et al 2008a).

Based on meta-analyses and large research studies, the sensitivity of the Pap test to identify CIN 2+ varies generally from 30% to 87% and specificity from 62% to 100% (Fahey et al. 1995, Nanda et al. 2000, IARC 2005). The pooled accuracy estimates derived from studies conducted in Europe and North America were 53% for sensitivity and 96.3% for specificity. Both sensitivity and specificity were reported to increase with increasing age (Cuzick et al. 2006). Very similar estimates were reported from the ATHENA study conducted in the U.S. Close to 47 000 women were screened by the LBC and the sensitivity was 52% and the specificity 93% (Castle et al. 2011).

8 265 women participated in a screening trial in Chile which is a middle-income developing country. The study used the infrastructure, personnel and protocols in place under the nationally organised cervical cancer screening programme. It showed that the verification bias-corrected sensitivity of a Pap test for CIN2+ was as low as 22.1%. The authors suggested that rapid population growth in the study area had overloaded the public health system and, thus, affected the Pap test quality (Ferreccio et al. 2012). This result emphasises the need for well functioning infrastructure and tight quality control in order to maintain high programme sensitivity when using conventional cytology (Arbyn et al 2008a, Lönnberg et al. 2010). In Finland within such settings, the cross-sectional sensitivity of conventional cytology to identify CIN 2+ cervical lesion has been reported to be as high as 83% and specificity 94% using the cut-off cytology equal to LSIL or worse (Nieminen et al. 2004).

6.3.3 Diagnosis and treatment of cervical neoplasia

Generally, the screening test is only suggestive for underlying disease and the diagnostic confirmation is always made with some other method. The gold standard assessment of cervical precancerous lesions and cancer is colposopy-directed punch biopsy in which the cervix, vagina and vulva are examinated with a binocular light microscope with up to 40-fold magnification (Anderson et al. 1996). The colposcopic examination of the cervix includes that of the squamous epithelium, the transformation zone, the squamocolumnar junction and the visible part of the columnar epithelium. During the colposcopy, a 3% to 5% acetic acid solution is applied at the cervix causing tissue swelling and coagulation of the superficial intracellular proteins. This can be observed as reduced transparency and whitening of the cervical epithelium, a phenomenon known as acetowhitening. Also the structures of blood vessels in the lesion and outside of it are observed. However, an aceto-white reaction does not necessarily indicate presence of cervical neoplasia as it may also occur in immature squamous metaplasia and in healing or regenerative cervical epithelium (Anderson et al. 1996, IARC 2005, Arbyn et al 2008a). From the most prominent areas of acetowhitening, punch biopsies are taken for histological confirmation.

Underwood et al. recently performed a systematic review and meta-analysis of 32 papers on the accuracy of the colposcopy-directed punch biopsies to diagnose high-grade CIN. The pooled sensitivity for a colposcopy-directed punch biopsy to diagnose CIN 2+ lesion was 91.3% (95% CI 85.3-94.9%) and the specificity was 24.6% (95% CI 16.0-35.9%). These, however, may overestimate the true performance due to verification bias, though (Underwood et al. 2012).

As a result of the variation in the accuracy of colposcopy, it has been proposed that multiple biopsies and also random biopsies in areas that look normal should be taken. Pretorius and colleagues demonstrated that the sensitivity of colposcopic biopsy for CIN 2+ was 57% and a random biopsy detected an additional 37% of CIN2+ lesions. The yield of CIN 3+ per colposcopy was greater when a colposcopically directed biopsy was augmented by up to four random biopsies in quadrants that appeared to be normal. However, high-grade lesions that were not visualised by expert colposcopists had thinner epithelium compared to biopsies from areas with abnormal impression in colposcopy (Pretorius et al. 2004).

The ASCUS-LSIL trial was a randomised clinical trial that compared three strategies for women with ASCUS and LSIL cytology: an immediate colposcopy, an HPV triage and a conservative management with repeat cytology (ALTS 2003). The sensitivity of an enrollment colposcopy with guided biopsy or biopsies to detect a 2-year cumulative CIN 3 or more severe lesion was 70%. The detection rate of CIN 3 increased significantly to over 80% with two or more biopsies (Gage et al. 2006).

Usually the precancerous lesions are treated with excisional procedures allowing histological verification from the removed tissue. In Finland, the most widely used treatment is a loop electrosurgical excision procedure (LLETZ or LEEP) in which excision of the transformation zone is done using a diathermy loop under colposcopical control. The Finnish Current Care guidelines recommend that CIN 2+ lesions are treated

immediately, but CIN 1 lesions should be managed with surveillance until regression or treated in the case of progression (Finnish Current Care guidelines 2010).

6.3.4 Adverse effects of screening

The detection of precancerous lesions gain benefit by preventing some of the lesions from progressing to invasive cancer. Inevitably, screening also leads to harm by detecting lesions that would never have progressed to invasive disease. However, they will be treated if diagnosed which results in overdiagnosis and overtreatment (Malila et al. 2013).

Unnecessary referrals for further examinations and diagnostic and treatment procedures are often considered as major adverse effects of screening (IARC 2005). Overmanagement is of particular importance for women of reproductive age because the rate of preterm deliveries and subsequent perinatal morbidity and mortality are associated with excisional treatments for precancerous lesions, especially when the cone depth exceeds 10mm (Kyrgiou et al. 2006, Jakobsson et al. 2007, Arbyn et al. 2008b, Simoens et al. 2012). Also, nowadays the most widely used LLETZ seems to increase the risk of preterm delivery regardless of the histopathologic diagnosis, and particularly repeat procedures should be avoided (Simoens et al. 2012, Heinonen et al. 2013). On the other hand, treatment for CIN does not impair fertility (Kalliala et al. 2012).

In addition to the possible increased health care costs brought about by the overdiagnosis and overtreatment of an individual, psychological and social adverse effects exist as well. These include anxiety and fear of cancer in women attending a colposcopy after an abnormal cervical smear (Hellsten et al. 2008). Feelings of stigma, shame and anxiety may rise even without a referral for colposcopy when women know to have a sexually transmitted HPV infection which is also a risk factor for cancer (Waller et al. 2007). The psychological and social burden of the infection may also have an effect on social and sexual relationships (McCaffery et al. 2006). A health care professional communicating the test result properly and emphasising that HPV is a common infection that affects most people during their lifetime can reduce these feelings (McCaffery et al. 2006, Waller et al. 2007).

The factors affecting adverse effects of screening are optimally controlled in an organised screening programme. They can be minimised by carefully planning for screening intervals and the age range of women covered by the programme, using an accurate screening test and by monitoring the quality of screening (Rebolj et al. 2007, Arbyn et al. 2008a).

6.4 Methods for the detection of HPV infection

As human papillomavirus cannot be cultured easily, HPV detection assays rely on the detection of viral nucleic acids, mostly viral DNA. Many test systems have been developed that can detect a broad spectrum of genomes of hrHPVs and their transcripts (IARC 2007, Arbyn et al. 2008a, Snijders et al. 2010, Poljak et al. 2012). To date, at least

125 distinct commercial HPV tests exist but only a small subset of them has documented clinical performance for any of the standard HPV testing indications. Furthermore, for more than 75% of the tests currently on the market, not a single publication in peer-reviewed literature can be found (Poljak et al. 2012). The molecular HPV detection methods that are currently in use can be divided into target (i.e. genomic) amplification and signal amplification methods (Snijders et al. 2010).

The different HPV assays display a different sensitivity and specificity to detect HPV presence, i.e. they have a different analytical sensitivity and specificity (IARC 2007). This is not only due to the differences in primers and probes, but also the variation in the sampling method, the specimen transport medium, the DNA purification, the PCR buffer and amplification conditions and the interpretation of results will all contribute to the difference in analytical sensitivity and specificity (Gravitt et al. 2008). Various HPV tests also differ in their performance of detecting an HPV-related preinvasive disease or cancer, i.e. they have a different clinical sensitivity and specificity (Snijders et al. 2003, IARC 2007, Meijer et al. 2009). A high analytical sensitivity (i.e. detection of very low viral copy numbers) is critical for accurate HPV detection and genotyping. However, for screening purposes the detection of HPV is not useful unless it indicates the presence or development of high-grade cervical neoplasia (CIN 2 or CIN 3) or cancer and, thus, the methods require high clinical sensitivity (Meijer et al. 2009). Thus far, only two of the detection methods, i.e. Hybrid Capture 2 and GP5+/6+-PCR-EIA have shown an optimal balance between clinical sensitivity and specificity. They have been clinically validated for cervical screening purposes without any limitations (Hesselink et al. 2006, Meijer et al. 2009, Snijders et al. 2010, Poljak et al. 2012).

6.4.1 Target amplification methods

Target amplification methods are based on copying a specific nucleic acid sequences up to a level that can be easily detected using one of the many read-out systems. Some of these methods do not distinguish individual HPV types at all, some include concurrent or reflex genotyping for HPV16 and HPV 18 or other main HPV types, and some allow individual determination of several HPV types (Poljak et al. 2012).

The most commonly used target amplification method is the polymerase chain reaction (PCR). In PCR, the repetitive cycles of temperature switches cause denaturation of DNA, annealing of primers (i.e. target specific oligonucleotides) and extension of them by a thermostable DNA polymerase. A major challenge was to develop PCR assays that could detect as many mucosal HPV types as possible during one assay run. This was accomplished by using consensus primers that are directed to highly conserved sequences of the L1 gene in the HPV genome (IARC 2007, Snijders et al. 2010). The most widely used consensus primers today are GP5+/6+ (de Roda Husman et al. 1995) and PGMY09/11 (Gravitt, Peyton et al. 2000).

Most often viral DNA is the target of amplification but also a messenger RNA (mRNA) can be used. HPV early proteins. Given the consistent overexpression of viral oncogenes E6 and E7 and their proteins in malignant tissues (zur Hausen 2002), these are

the most relevant transcripts for diagnostic assays. The detection of viral mRNA can be done by reverse-transcriptase PCR or by nucleic acid sequence-based amplification (NASBA) (IARC 2005). The presence of viral mRNA transcripts might be a more specific predictor of persistent and progressive infection than the presence of viral DNA (Cuschieri et al. 2004b). However, due to the lack of existing research on the topic and the considerable clinical heterogeneity a systematic review could not make a solid conclusion about clinical applicability of HPV mRNA testing (Burger et al. 2010). Two HPV-E6 tests are currently under validation studies (Cuzick et al. 2012).

Several read-out systems to detect PCR products exist. Most of them are based on hybridisation methods using labelled primers and / or antibodies so that the hybrids can be visualised via colorimetric or fluorescent staining procedures (IARC 2007, Snijders et al. 2010). One of the well-known read-out systems is the Enzyme Immunoassay (EIA) in which biotin-labelled consensus primers are used for PCR amplification. Then biotinylated HPV amplicons are captured onto a streptavidin-coated microwell plate and hybridised with digoxigenin-labelled HPV type-specific oligoprobes (Jacobs et al. 1997, Kornegay et al. 2001). If used for genotyping, the EIA is laborious and requires a large amount of PCR products and, thus, reverse hybridisation techniques, such as the Luminex technology, have been introduced. This microarray technology is based on polystyrene beads that are dyed with various ratios of two spectrally distinct fluoropheres and their unique spectral signatures are recognised by Luminex analyser (Schmitt et al. 2006).

6.4.2 Signal amplification methods

Signal amplification methods are based on the hybridisation of nucleid acids with target-specific probes, either in liquid phase or *in situ* on cells or tissue. The signal describing the hybridisation event is amplified and visualised with one of the various available methods (Snijders et al. 2010).

The most widely used signal amplification method and the most frequently used HPV test worldwide is the HC2 test (Snijders et al. 2010, Poljak et al. 2012). HC2 is a commercial product that uses a mixture of synthetic RNA probes which are complimentary to genomic sequences of 13 hrHPV genotypes including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 and therefore hybridise to viral HPV DNA in samples. DNA-RNA hybrids are then captured in microplate wells by specific antibodies and HPV DNA is detected by a series of reactions that yields a light signal that can be measured in a luminometer (IARC 2007, Arbyn et al. 2008a, Snijders et al. 2010). The intensity of the emitted light, expressed as Relative Light Units, is related to the amount of viral DNA and provides a semiquantitative measure of the viral load in the specimen (IARC 2007, Arbyn et al. 2008a). HC2 was the first HPV DNA test that was approved by the Food and Drug Administration (FDA) in the USA in 2003. It has been evaluated extensively in clinical trials and cohort studies ever since. New HPV tests should show that they possess clinical characteristics equivalent to the HC2 before they can be adapted for cervical screening purposes (Meijer et al. 2009). All FDA-approved HPV tests and their characteristics are given in Table 3.

Table 3. *FDA-approved commercial HPV tests which can be used to detect hrHPV DNA**

HPV test	Manufacturer	Targets	Distinction of HPV16/18	FDA-approved indications
37				
Hybrid Capture 2	QIAGEN Inc. Gaithersburg, MD, USA	DNA, 12 hrHPV types + HPV 68	No	screening of women ≥ 30 years adjunct to cytology triage in women with ASCUS cytology
Cervista HPV HR	Hologic Madison, WI, USA	DNA, 12 hrHPV types + HPV 66, 68	No	screening of women ≥ 30 years adjunct to cytology triage in women with ASCUS cytology
Cervista HPV 16/18	Hologic Madison, WI, USA	DNA, HPV 16, 18	Yes	reflex test for Cervista HPV HR positive women; presence of HPV 16 and 18 in adjunct to cytology triage in women with ASCUS cytology
cobas	Roche Molecular Systems Inc. Alameda, CA, USA	DNA, 12 hrHPV types + HPV 66, 68	Yes	screening of women ≥ 30 years adjunct to cytology triage in women ≥ 21 years with ASCUS cytology presence of HPV 16 and 18 in women ≥ 21 years with ASCUS cytology presence of HPV 16 and 18 in women ≥ 30 years adjunct to cytology
Aptima	Gen-Probe Inc. San Diego, CA, USA	mRNA, 12 hrHPV types + HPV 66, 68	No	screening of women ≥ 30 years adjunct to cytology triage in women with ASCUS cytology

*12 hrHPV types based on the classification by the International Agency for Research on Cancer (IARC) Group 1: (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59)

6.5 The accuracy of HPV-based screening

There are three generally accepted purposes for HPV DNA testing in cervical cancer prevention: 1) triage of women with equivocal or low grade cytologic abnormalities; 2) prediction of a therapeutic outcome after treatment of CIN; and the scope of this thesis 3) primary screening for cervical cancer. The accuracy of HPV-based screening can be assessed by parameters of sensitivity, specificity, positive and negative predictive values, referral rate and a risk of cancer (or severe precancerous lesion). Sensitivity measures the screening test's ability to correctly identify an underlying disease whereas specificity refers to the screening test´s ability to correctly identify an individual who does not have any disease. Absolute parameters can be obtained if a sufficient number of study subjects are diagnosed using the gold standard verification method i.e. a colposcopy combined with a follow up for the detection of possible development of invasive cancer in the future. However, it is not often possible to assess absolute sensitivity or specificity as those women with negative screening test results are not usually referred to histological verification. This means that an underlying disease may not be discovered in women who test negative by the screening test. In that case, relative parameters can be assessed from cross-sectional studies and randomised controlled trials (RCTs) by comparing two screening tests and using surrogate outcomes as a measure of future invasive cervical cancer (IARC 2005).

6.5.1 Cross-sectional performance

Numerous studies have reported cross-sectional performance of HPV DNA testing in comparison with conventional or liquid based cytology. The results of these studies are summarised in a few comprehensive systematic review articles and meta-analyses. There is indisputable evidence that HPV testing is more sensitive for detecting cervical precancerous lesions than cytology. The cross-sectional sensitivity of the HPV DNA test alone for detecting high-grade cervical lesions is, on average, 20-40% higher but the specificity is 3-10% lower than that of cytology, depending on the outcome of interest (CIN 2+ or CIN 3+) and the threshold of cytology (ASCUS or LSIL) (Arbyn et al. 2006, Koliopoulos et al. 2007, Cuzick et al. 2008, Zhao et al. 2010, Whitlock et al. 2011, Arbyn et al. 2012b). The specificity of the HPV DNA test alone has shown to increase with age (Kulasingam et al. 2002, Cuzick et al. 2006).

Positive Predictive Value, or Precision Rate, is a critical measure of the performance of the screening test as it describes the proportion of individuals with positive test results that have the disease they are tested for. PPV is dependent on the prevalence of the disease (or outcome of interest) in the population. In terms of cervical cancer screening, highest PPVs are seen at younger screening age groups (Sherman et al. 2003, Cuzick et al. 2006). As the great majority of HPV infections are transient and therefore are not associated with severe cervical lesions, the positive predictive value of HPV DNA test alone is poor and substantially lower than that of cytology (Naucler et al. 2009).

HPV DNA test has a very high negative predictive value meaning that when the test yields a negative result, it is most likely that the person does not have cervical cancer or precancerous lesion. A low cumulative incidence of CIN 3+ and cancer following a negative HPV DNA test result has been observed in cohort studies. The joint study of 24 295 women from seven HPV studies conducted in six European countries reported the cumulative incidence of CIN 3+ after six years to be 0.27% (95% CI 0.12-0.45%) among HPV-negative women and 0.97% (95%CI 0.53-1.34%) among women with negative cytology at the screening visit (Dillner et al. 2008). A very large Kaiser Permanente cohort of 331 818 women aged 30 years and older reported the 5-year cumulative incidence of cervical cancer to be 7.5 per 100,000 women-years after a negative result in Pap test whereas 3.8 per 100,000 women-years after a negative result in HPV test and only slightly lower among women who tested negative for both HPV and Pap tests (3.2 per 100,000) (Katki et al. 2011). A negative HPV DNA test result alone or in combination with cytology assures a longer protection against future invasive cancer and CIN 3+. This means that screening intervals among HPV-negative women can be safely extended to be between five to ten years. The need for less frequent testing will naturally translate into significant cost savings of public fund (Dillner et al. 2008, Kjaer et al. 2010, Katki et al. 2011, Kitchener et al. 2011). Anyway, these extended screening intervals may not be appropriate in high-risk populations with higher HPV acquisition rates. In a low-income area in Brazil, the cumulative incidence of CIN 2+ lesions increased dramatically after 6 to 7 years among women with negative HPV DNA test at a baseline (Chevarie-Davis et al. 2013).

6.5.2 Randomised Controlled Trials on HPV screening

In addition to the previously conducted ones, there are ongoing Randomised Controlled Trials (RCTs) on HPV DNA testing in population-based screening programmes across the world. The settings and protocols of these trials are summarised in Table 2. RCTs have confirmed higher relative sensitivity of HPV DNA testing except for the Indian and the ARTISTIC trials which did not show increased detection for CIN 2+ and CIN 3+ lesions at a baseline (Tables 4 and 5). The similar rates in the ARTISTIC trial may have resulted from overdiagnosis of regressive lesions in the LBC group due to a low threshold for an abnormal cytology (up to 13% of all samples) (Kitchener et al. 2009). Incidentally, the HPV FOCAL study in British Columbia in fact showed a smaller number of CIN 2+ lesions in the HPV arm than in the control arm at the initial screening visit. However, as a result of the follow-up of HPV-positive women, the amount of colposcopies and CIN 2+ and CIN 3+ lesions increased substantially during the first screening round in the HPV arm (Ogilvie et al. 2012).

Table 4. *Accuracy of HPV-based screening in comparison with cytology screening at the baseline in the HPV screening trials in Europe and North America*

Trial (country)	CIN 2+			CIN 3+			References
	Relative sensitivity	Relative specificity	Relative PPV	Relative sensitivity	Relative specificity	Relative PPV	
ATHENA (U.S.)	1.72	0.96	1.05*	1.74	0.96	1.08*	Castle et al. 2011
POBASCAM (The Netherlands)	1.02*	0.98	1.04*	1.02*	0.98	1.01*	Bulkmans et al. 2004
CCCaST (Canada)	1.71	0.97	0.90*	N/A	N/A	N/A	Mayrand et al. 2007
NTCC (Italy)† Phase 1, 25-34y	1.61	0.91	0.55	0.70*	0.91	0.24	Ronco et al. 2006a
NTCC (Italy) Phase 1, 35-60y	1.47	0.98	0.40	1.25*	0.98	0.34	Ronco et al. 2006b
NTCC (Italy)† Phase 2, 25-34y	3.50	0.94	0.89*	2.61	0.93	0.66*	Ronco et al. 2008
NTCC (Italy)† Phase 2, 35-60y	1.92	0.98	0.80*	2.06	0.98	0.86*	Ronco et al. 2008
Swedescreen (Sweden)‡	1.34	0.96	0.45	1.30	0.95	0.44	Naucler et al. 2009

* Not statistically significant

† Including repeat tests of cytology performed until the woman either returned for a routine screening or was referred to a colposcopy and all histology diagnosis given within one year after the referral to the colposcopy. No evidence of heterogeneity on estimates between Phases 1 and 2

‡ Estimates for the HPV DNA test alone

Table 5. *Relative detection rates of HPV-based screening in comparison with cytology screening during the first and the second screening rounds of the RCTs in Europe or North America*

Trial (country)	Relative detection rate during 1st screening round		Relative detection rate during 2nd screening round		Round 1 plus round 2 (cumulative rates)		References
	CIN 2+	CIN 3+	CIN 2+	CIN 3+	CIN 2+	CIN 3+	
ARTISTIC (UK) *	1.14 (0.94-1.38)	0.97 (0.75-1.25)	0.63 (0.42-0.96)	0.53 (0.30-0.96)	0.99 (0.83-1.19)	0.85 (0.67-1.08)	Kitchener et al. 2009
POBASCAM (The Netherlands)	1.25 (1.05-1.50)	1.15 (0.92-1.43)	0.88 (0.71-1.08)	0.73 (0.55-0.96)	1.08 (0.94-1.24)	0.96 (0.81-1.14)	Rijkaart et al. 2012a
HPV FOCAL (Canada)	1.46†	1.60†	N/A	N/A	N/A	N/A	Ogilvie et al. 2012
NTCC (Italy) Phase 1 age 35-60‡	1.94 (1.40-2.68)	1.85 (1.16-2.95)	0.74 (0.34-1.62)	0.72 (0.23-2.28)	1.68 (1.25-2.26)	1.61 (1.05-2.47)	Ronco et al. 2010
NTCC (Italy) Phase 2 age 35-60‡	2.13 (1.50-3.03)	2.40 (1.43-4.05)	0.30 (0.11-0.81)	0.30 (0.08-1.11)	1.64 (1.18-2.24)	1.70 (1.08-2.67)	Ronco et al. 2010
Swedescreen (Sweden) ‡	1.51 (1.13-2.02)	1.31 (0.92-1.87)	0.58 (0.36-0.96)	0.53 (0.29-0.98)	1.17†	1.04†	Naucler et al. 2007b Naucler et al. 2009

* Odd ratios (ORs) from an unconditional logistic regression.

† Not reported in the original publication. Rates were calculated from the results available in the study. Significant differences between screening methods were observed in the HPV FOCAL study but not in the Swedescreen.

‡ CIN 2+ includes CIN 2, CIN 3 and AIS and CIN 3+ includes CIN 3 and AIS.

So far, two of the RCTs have reported a decreased risk of cervical cancer in the second screening round of HPV screening compared to cytology screening (Ronco et al. 2010, Rijkaart et al. 2012a). Also, the meta-analysis from randomised trials showed a notable reduction (RR 0.13; 95% CI 0.04-0.44) in the relative detection rate of cervical cancer in the second screening round among women who were HPV-negative in comparison with cytology-negative women at a baseline (Arbyn et al. 2012b). The Indian cluster-randomised study is different from other HPV-based screening trials as it used a 'once-in-a-lifetime' -approach and was conducted in a low-resource setting. However, it is the only study that has demonstrated a reduced mortality after HPV DNA testing compared to cytology during eight years of follow-up (Sankaranarayanan et al. 2009).

RCTs have confirmed a very high NPV of HPV DNA test. They have demonstrated over a 50% reduction in CIN 3+ lesions detected in the second screening round in women who were HPV-negative compared to cytology-negative at the initial screening visit (Arbyn et al. 2012b).

Taken into account the cumulative detection rates over the two screening rounds, RCTs have shown that the higher sensitivity of the HPV DNA test detects high-grade cervical lesions earlier. Detection rates of CIN 3+ were reduced, but not significantly, in the 2nd screening round, and the total rates over the two screening rounds were increased in the NTCC study Phase 2 involving the most aggressive protocol (i.e. an immediate colposcopy) for HPV DNA-positives (Table 5). RCTs have also demonstrated that an increased amount of CIN 2 lesions diagnosed at the first screening is not always accompanied with a statistically significant reduction in CIN 2 lesions at later screenings (Naucler et al. 2007b, Ronco et al. 2010, Rijkaart et al. 2012a) indicating that the higher sensitivity of HPV-based screening may result in an excessive detection of non-progressive CIN 2 lesions. This was most pronounced during Phase 2 of the NTCC trial when HPV-positive women aged 25-34 years old were referred to an immediate colposcopy regardless of cytology. That protocol resulted in a relative detection rate of 4.54 (95% CI 2.95-6.99) for CIN 2+ and 4.00 (95% CI 2.07-7.73) for CIN 3+ if women were screened by the HPV DNA test in comparison with cytology (Ronco et al. 2010).

6.5.3 Co-testing

Co-testing is marginally more sensitive but clearly less specific than testing by the HC2 or cytology alone (Cuzick et al. 2008, Whitlock et al. 2011, Arbyn et al. 2012b). With regard to a successful implementation of a screening test for routine practice, the acceptability of the test among population and the feasibility of running the programmes play substantial roles. In the U.S. the new guidelines for cervical cancer screening and prevention has been published in 2012. The most recommended method for women aged 30 years and older is currently co-testing every five years which can be seen as a huge development from the previous annual Pap testing (Saslow et al. 2012). Also in some high risk populations, the best optimisation of sensitivity and specificity may be achieved by using dual testing (Chevarie-Davis et al. 2013).

6.6 Triaging of HPV-positive women

A positive hrHPV test result does not necessarily indicate an immediate need of colposcopy as most infections are transient and do not cause a significant cellular atypia. Several strategies have been suggested to improve specificity of the HPV DNA testing during primary screening. The simplest method is to repeat the HPV test after a year to identify a persistent infection which is needed for the development of precancerous lesions and cancer (Arbyn et al. 2008a). It should be noted, however, that this strategy is limited by the fact that some low-risk HPV types may persist but still never cause a neoplastic progression (Schiffman et al. 2005).

The diagnostic threshold of RLU 1.00 of the HC2 test was based on the data obtained from the clinical materials. Increasing the cut-off from 1.0 to 2.0 or 3.0 relative light units (RLU/Co ratio) would result in a significant reduction in test positivity related to normal and borderline/mild cytological abnormalities but only in a negligible decrease in sensitivity (Cuzick et al. 2003, Hesselink et al. 2006, Ronco et al. 2006a, Sargent et al. 2010). For screening purposes even using the RLU/Co ratio $\geq$ 10.0 results only in a marginal loss in detection of precanerous lesions but improves the specificity of the HPV test substantially (Kotaniemi-Talonen et al. 2008b).

To reach the full potential of HPV testing, new approaches with better specificity are needed. Either as triage tests for HPV-positive women or possibly even as alternative screening modalities (Cuzick et al. 2012). Of these approaches, the detection of viral oncogene transcripts (mRNA) was described previously (section 7.4.1). Available triage tests include cytology and new cytologic methods such as immunocytological stainings, HPV typing and methylation of host genes.

6.6.1 Cytology

A Pap smear following a positive HPV result has been seen as an attractive screening strategy in countries where cytology is of good quality (Cuzick et al. 2008, Kotaniemi-Talonen 2008a, Naucler et al. 2009, Rijkaart et al. 2012b).

The sensitivity of co-testing using the HPV DNA test and cytology is virtually the same as using the HPV DNA test alone (Cuzick et al. 2008, Schiffman et al. 2011, Whitlock et al. 2011, Arbyn et al. 2012b). Also, the marginal gain in sensitivity is followed by a substantial number of screen positive women who need further examinations or repeat screenings (Castle et al. 2011). However, by conducting the more sensitive HPV DNA test first and subsequently the Pap test as a triage for HPV-positives reduces the amount of screening tests required and is, therefore, a more economical and cost-effective way of testing (Kotaniemi-Talonen et al. 2005, Kotaniemi-Talonen et al. 2008a, Naucler et al. 2009, Schiffman et al. 2011). Also, a low PPV of a positive HPV DNA test to identify presence of CIN 2+ can be raised to the level of conventional cytology by using the cytology triage (Kotaniemi-Talonen et al. 2005).

A post-hoc analysis of 14 triages or follow-up strategies from the POBASCAM screening trial concluded that triage hrHPV-positive women with cytology followed by

repeat cytology at 12 months would be the preferred strategy. This yielded the best balance between the benefits measured through a high NPV of 99.3% and the harms measured through a modest colposcopy referral rate (33.4%; 95% CI 30.2-36.7%) (Rijkaart et al. 2012b).

The limitation of cytology as a triage is that HPV-positive women remain at a substantial risk for CIN 3+ even after a negative cytology triage result (Sherman et al. 2003, Dillner et al. 2008, Katki et al. 2011) and therefore require some kind of an intensified follow-up before the resumption of routine screening (Schiffman et al. 2011).

6.6.2 Genotyping

The risk of CIN 3 or cancer differs substantially between the different hrHPV types. Women with the HPV 16 infection are considered being at the greatest risk (Sherman et al. 2003, Schiffman et al. 2005, Khan et al. 2005b, Naucler et al. 2007a, Kjaer et al. 2010). Thus, genotyping has been suggested as an alternative to cytology in triaging an HPV-positive result and stratifying women to a more appropriate management strategy.

Genotyping has also been proposed to be used adjacent to cytology among HPV-positive women with a normal cytology. Women that are HPV 16 or HPV 18 -positive, could then be subjected to colposcopy or to a more intensive follow-up (repeat test at 6 to 12 months). Others could be managed with a repeated HPV test that is carried out in 12 to 24 months (Meijer et al. 2006, Huh et al. 2010, Cuzick et al. 2012). Interestingly, cancers that are diagnosed following HPV+/cytology- results often have a glandular component which suggests that the screening algorithm of HPV+/cytology- women should perhaps also include an endocervical curettage (ECC) to search for adenocarcinoma (Kinney et al. 2011).

6.6.3 Other biomarkers

Most of the biomarkers identified so far indicate HPV-associated transformation which, in turn, has led to lesion progression. These biomarkers measure an increased HPV oncogene expression, an increased cell proliferation or chromosomal instability and they are more prevalent in CIN 3 lesions than in HPV infection (IARC 2005, Schiffman et al. 2011).

One interesting biomarker for HPV-positives at the moment is immunocytochemistry staining of cytology slides for p16-INK4A (Schiffman et al. 2011, Cuzick et al. 2012). The overexpression of the cyclin-dependent kinase inhibitor p16-INK4A is associated with the disruption of the retinoblastoma cell cycle pathway by HPV E7 and the proportion of cervical smears overexpressing p16-INK4A has been shown to increase with the grade of atypia in cytology. Based on a meta-analysis, 12% of normal Pap smears were positive for the biomarker compared to 45% of ASCUS and LSIL and 89% of HSIL smears (Tsoumpou et al. 2009). A nested substudy of the NTCC randomised HPV screening trial demonstrated that the relative sensitivity of an HPV testing and p16-INK4A triage versus conventional cytology for CIN 2+ was 1.53 (95% CI 1.15-2.02) with

no substantial increase in referral to colposcopy (RR 1.08; 95% CI 0.96-1.21) (Carozzi et al. 2008). The recently introduced morphology-independent dual-stain protocol, that detects simultaneously p16-INK4A and Ki-67 expression in cervical cytology samples, provided comparable sensitivity but higher specificity for high-grade cervical lesions than p16 single-stain cytology (Petry et al. 2011, Schmidt et al. 2011, Cuzick et al. 2012).

DNA methylation (DNAme) is an essential epigenetic modification of the genome that relates to the regulation of many cellular processes. An aberrant DNAme can lead to altered transcription, chromosomal instability, cell immortality and malignant transformation. The DNAme generally leads to the silencing gene expression and, thus, various established or candidate tumor suppressor genes are mechanistically the most relevant for the development of a tumor (Robertson 2005, Cuzick et al. 2012).

The aberrant methylation pattern is detectable in cervical smears years before the diagnosis of cervical cancer thus suggesting that DNAme might serve as a triage tool for hrHPV-positives (Cuzick et al. 2012). A panel including two tumor suppressor genes involved in cervical carcinogenesis (CADM1 -cell adhesion molecule 1 and MAL -T-lymphocyte maturation associated protein) was assessed and validated as a potential triage test in a population-based cervical screening setting in the Netherlands. The panel was equally discriminatory for CIN 3+ as cytology or cytology with HPV 16 or HPV 18 genotyping in hrHPV-positive women. For the outcome of CIN 2+, cytology and the combination of cytology and HPV 16 or HPV 18 genotyping performed slightly better than the methylation panel. This may indicate that CIN 2 lesions represent productive hrHPV infections with a low progression potential until the next screening round in five years´ time (Hesselink et al. 2011).

7 AIMS OF THE STUDY

The study was a randomised trial on public health services conducted within the organised cervical cancer screening programme in Finland. The aim of this study was to assess the prevalence of cervical hrHPV infections in Finland and to evaluate the use of an HPV DNA test in cervical cancer screening. The performance of a primary HPV DNA testing was evaluated both in a cross-sectional and in a prospective setting compared with conventional cytology.

The specific studies included within this thesis had the following more detailed aims:

1. To assess the prevalence of the hrHPV infection and associated sociodemographic factors in the Finnish screening population and to compare the prevalence with other European countries in an age-specific manner.

2. To assess the age-related performance and validity of the screening method by comparing screening by primary HPV DNA test (with or without cytology triage) with screening by conventional cytology. Cross-sectional parameters of interest: test positivity, recommendation to intensive screening, referral to colposcopy, histological detection rates, relative sensitivity, relative specificity and positive predictive value at the index (first) screen visit.

3. To assess the effect of screening method on the burden of cervical precancerous lesions and cancer over one screening round comparing prospective detection rates of primary HPV screening with cytology triage to those of conventional cytology.

4. To assess the distribution of hrHPV types in the Finnish screening population and to determine the hrHPV types that cross-sectionally relate to cytological and histological cervical abnormalities.

The results from these evaluations are essential when considering for a switch to an HPV-based cervical cancer screening programme, for tailoring HPV-based screening algorithms and also for planning for, and monitoring the impact of an HPV vaccination.

8. Material and methods

8.1 The organised cervical screening programme in Finland

An organised population-based programme for cervical cancer screening was introduced in a small number of municipalities in Finland in 1963. Following the initial pilot areas, the programme had expanded nationwide by the early 1970s (Anttila and Nieminen 2000, Anttila and Nieminen 2007). The Government Decree on Screenings (339/2011) states that screening is a part of preventive health care which municipalities shall organise for their inhabitants. Screening for cervical cancer must be offered for all 30 to 60-year-old women, every five years, free of charge. Some municipalities start to invite woman already at the age of 25 and / or continue to do so up to the age of 65. In the current thesis the results are given using age groups because of some minor fluctuations in the screening policies within the different municipalities. This is despite the fact that the majority of the screening participants were exactly of the age as per Government guidelines i.e. 30, 35 and so forth.

The Population Information System is used to identify the women in the screening target population which results in an invitational coverage of 99.0% among target age groups (Finnish Cancer Registry 2009). Women are invited for screening by personal letter and approximately 70% of them attend, with the attendance rate ranging from 56% among the 30-year-olds to approximately 78% among women aged 60 or older (Finnish Cancer Registry 2009). The screening interval is five years unless intensive screening is indicated due to a previous abnormal screening test result or reported bleeding symptoms at the screening visit. The screening test in nationwide use has been conventional cytology since the introduction of the screening programme.

It has been estimated that around 450 000 Pap-smears are taken annually in Finland for screening purposes i.e. among women who do not have any abnormal bleeding or any other gynaecological symptoms. Only about one third of the annual Pap-smears are taken as a part of the organised screening programme. This means that substantial amount of cervical precancerous lesions are detected every year outside the organised screening programme (The National Institute for Health and Welfare 2011).

8.1.1 Conventional screening protocol

At a screening visit a trained nurse or midwife makes a gynaecological anamnesis utilising a structured information form and also takes the screening sample. A conventional Pap smear consists of three subsamples including vaginal, cervical and endocervical cells. The information forms and fixed samples are then sent to screening laboratories in which cytological smears are stained and interpreted. Primary interpretation of the Pap smear is performed by a trained cytotechnician. A cytopathologist reviews all the abnormal findings and also the subsets of normal findings.

The screening results are given to all participants within a month from the screening visit. If the results are normal, or are only slightly abnormal indicating a recommendation for intensive screening (i.e. re-screening within 12 to 24 months), the results are given by letter. If, however, a need for further examinations is indicated, the most frequently by way of colposcopy, the women are informed by phone instead.

In the current thesis, cytology was reported using the modified Papanicolaou classification system, including descriptive diagnosis, in 2003-05 and the Bethesda System (TBS) year 2001 version (Solomon et al. 2002) thereafter. Women who had a squamous intraepithelial lesion of a low to a more severe grade (ACH-H, LSIL, HSIL and AGC-FN in TBS 2001) and an earlier Papanicolaou class of III–V in cytology, was immediately referred to a colposcopy and biopsy. ASCUS and AGC-NOS with an earlier Papanicolaou class II, was interpreted as a borderline result. This indicated a need for intensifive screening within the screening programme unless an immediate colposcopy was recommended by the cytopathologist e.g. due to two or three consecutive borderline cytology result. Women who were referred to colposcopy but do not have a histologically confirmed CIN were also targeted for the intensive screening.

In Finland the general practice is that colposcopies are made and the biopsy specimens are analysed in local hospitals based on routine procedures. During the first three years of the study, all cervical lesions regardless of their grade (CIN 1+) were treated, usually with the LLETZ. Since 2006 onwards, Finnish guidelines have recommended that CIN 2+ lesions are treated immediately, but women younger than 30 years with CIN 1 lesions should be managed with surveillance until regression or treated if progression. This management strategy of surveillance was expanded in 2010 to cover all CIN 1 lesions, independent of the woman's age (Finnish Current Care Guidelines 2010). However, clinical practices might have varied regardless of the recommendations.

8.2. Randomised implementation of HPV DNA screening

Primary screening with an HPV DNA test was launched as a randomised public health policy within the national organised cervical cancer screening programme in 2003. In the study area, screening visit procedures are not changed from the preceding routine in the control arm. In the experimental arm, a commercial HPV DNA test (Hybrid Capture 2) is offered as a primary screening test and cytology triage with a conventional Pap smear is provided to women with positive HPV DNA results. In the beginning, there were seven municipalities of Southern Finland (Hyvinkää, Järvenpää, Kirkkonummi, Lohja, Porvoo, Tuusula and Vantaa) and one screening laboratory (the FCO laboratory in Helsinki) involved. The next year two more municipalities (Espoo and Helsinki) and one screening laboratory (HUSLAB in Helsinki) joined the study.

The National Authority for Medicolegal Affairs permitted that written informed consent from women participating in HPV DNA screening was not needed because the trial was regarded as routine practice and involved a very large number of women. Also the results of the HPV DNA screening, which were needed for making decisions regarding follow-up and management, were allowed to be registered in the screening database.

Screening protocol and data collection was approved by the Ethical Committee of Obstetrics and Gynecology in Hospital District of Helsinki and Uusimaa, and also by the Health Boards of the committed municipalities before the onset of the screening trial. The nurses and midwifes, who took the screening samples, were trained for a half-day for technical issues concerning an HC2 sampling and they were also educated to inform the attending women of the HPV infection and the probable consequences of the HPV DNA screening. Laboratory personnel in the screening laboratories were trained for an HC2 analysis for three days by the manufacturer´s representative.

8.2.1 HPV-based screening protocol

Women of selected municipalities are individually randomised (1:1) to undergo primary screening with the HPV DNA test or to undergo conventional cytology screening (Pap test) using computer generated random numbers. Women in both study arms are invited for screening using a similar invitation letter with general information on screening. Using a special written material provided in the letter or at screening laboratories, women are informed of a new screening test in use that is at least as reliable as the Pap test. By the protocol, the randomisation status is not shown in the invitation letter. The assigned test method is disclosed and discussed during the screening visit and then woman can decline the HPV test and get the routine Pap test instead.

From women who are allocated to the HPV DNA screening arm, vaginal and cervical samples for the Pap smear are prepared as in the routine practice. However, endocervical cells are collected with the special brush included in the HC2 assay instead of a regular cytobrush. The brush is first used to prepare a cytological smear and after that placed into a tube containing standard transport medium (STM). The tube is then delivered to the screening laboratory for further processing and analysis.

The transport medium containing the endocervical cells is subsequently processed with the supplies and reagents of the Hybrid Capture 2 assay. A probe mixture that detects 13 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) is used according to the manufacturer's instructions. The measurement of the HPV DNA is expressed as a ratio of Relative Light Units (rlu ratio) to the average of three positive controls. A cutoff for a positive hrHPV DNA result is defined as an RLU ratio of 1.00 or higher (equivalent to HPV DNA concentration of 1 pg/mL) and an RLU ratio of less than 1.00 is considered HPV DNA–negative.

Staining and interpretation of cytological slides in the HPV DNA screening arm is done in a conventional manner but cytology triage only follows if 1) the woman´s HC2 test result is positive 2) the HPV DNA sample is not available due to technical errors or the woman´s refusal and 3) the woman reports abnormal bleeding symptoms during the screening visit. Cytological criteria for defining abnormalities are the same as in the conventional (control) arm. Cytotechnicians and the cytopathologists are aware of the HPV DNA test result. Colposcopies and other further examinations are performed in a routine manner and histological criteria follow those in the conventional screening arm.

Also colposcopists and pathologists involved in diagnostic procedures in local hospitals have access to the woman's medical records, including their screening test results.

The management of HPV-positive women is based on the cytology triage result in the first place. In case that cytology triage is normal, an invitation to re-screening (intensive screening) after 12 to 24 months is indicated, however. During the intensive screening, women with persistent HPV infections are identified and referred to the colposcopy after two or three consecutive positive HPV DNA test results.

8.3 Data sources and their linkage

8.3.1 The Screening Register (I-IV)

The Mass Screening Registry of the Finnish Cancer Registry receives routinely reports on all screened women in Finland and this data collection is ensured by law. The data is registered at an individual level using unique personal identifiers. The registered data includes information on the invitation (year, municipality and reason for invitation) and on the screening visit (screening laboratory, time of screening visit, result of screening test and recommendations for management). The screening information also contains gynaecological anamnesis including possible abnormal bleeding symptoms, history of gynaecological treatments and number of births and / or abortions. In case that further diagnostic examinations (mainly colposcopy) are indicated, records also include the date and result of histological verification.

Histological diagnoses in the MSR database were registered according to the WHO classification and converted to three-tier classification for CIN, in which CIN 1 equals mild dysplasia, CIN 2 moderate dysplasia, and CIN 3 severe dysplasia and carcinoma *in situ* (Figure 3). During the study period, the screening register did not make any distinction between squamous and adenomatous lesions. Thus, CIN 3 also includes adenocarcinoma *in situ* (AIS) cases. Moreover, invasive cervical cancers that are derived from the MSR database include both squamous cell carcinomas and adenocarcinomas. For the study II, only histological diagnoses in the screening database of the MSR were used. Information on the screening visit is fairly complete in the screening database but data on cervical lesions and cancers detected afterwards, e.g. in subsequent screening episodes among women with borderline screening test result, may remain incomplete. The current thesis is based on the routine data restricted to the time period since 2003 which is when randomised HPV screening trial was implemented into the screening programme.

8.3.2 The Cancer Register (III and IV)

The Finnish Cancer Registry (FCR) was founded in 1952 by the Cancer Society of Finland. It collects and monitors cancer data at an individual level, produces cancer

statistics and provides data for research purposes. Cancer notifications are obtained from hospitals, physicians and pathological and haematological laboratories. In addition, Statistics Finland sends information on all death certificates with cancer as a cause of death every year. Due to different information sources and active work in reviewing and compiling the data, the cancer register is a virtually complete database with regards to invasive disease (Teppo et al. 1994, Lönnberg et al. 2011).

Diagnostic coding of the cancer register is based on the 3rd edition of the International Classification of Diseases for Oncology (ICD-O-3) (Fritz et al. 2000). The data covers diagnostic details (date of diagnosis, cancer site, morphology, stage and behaviour), information on primary treatment, and the date and cause of death, if applicable. Besides all malignant neoplasms, reporting of some precancerous lesions of the cervix uteri (ICD-O-3 topography codes C53.0 – C53.9) to the FCR is obligatory. These are defined as CIN3 or AIS in the current study and include CIN 3 with ICD-O-3 morphology code 8077/2, squamous cell carcinoma *in situ* (8070/2), epithelial neoplasm (not otherwise specified) *in situ* (8010/2) and adenocarcinoma *in situ*, AIS (8140/2). Furthermore, the diagnosis of dysplasia gravis is registered with a separate in-house code as it is not specified in ICD-O-3. Cervical precancerous lesions have been historically under-reported but since the late 1990s figures are likely to reflect a true incidence of these lesions (Finnish Cancer Registry 2009).

The current thesis utilise invasive cervical cancers diagnosed since 1964 and cervical precancerous lesions since 2003. When the data sources were combined, the cancer data was available up to year 2008.

8.3.3 Care Registers for Social Welfare and Health Care (III and IV)

Care Registers for Social Welfare and Health Care (formerly the Finnish Hospital Discharge Register, HDR) includes data from inpatient episodes and day-surgical procedures, the former since 1969 and the latter since 1994. Starting from 1998, the data collection appended to include all outpatient visits in the public sector. In the HDR, every contact to the healthcare service is recorded with some diagnosis based on ICD-10 diagnosis codes (from 1996 onward). The data also includes the date of visit or discharge and the procedures done for diagnostic or treatment purposes.

Data collection from the health service providers is ensured by legislation. However, some of the registered data is mandatory report whereas other parts are based on voluntary actions which have an effect on the coverage and usability of the information. From the HDR we asked for all the healthcare contacts recorded with ICD-10 diagnosis codes of cervical intraepithelial neoplasia (N87.0, N87.1, N87.2 and N87.9), cervical carcinoma *in situ* (D06) and invasive cervical cancer (C53). N87.9, which is used for a dysplasia, not otherwise specified, was considered as CIN 2 in our study. The current thesis is based on the HDR data restricted to the years 2003–2008.

8.3.4. The Population Information System (I-IV)

The Finnish Population Information System is a national register. It contains basic information related to the identification of Finnish citizens and foreign citizens residing permanently in Finland. Personal data recorded in the system includes name, date of birth, personal identity code, address, citizenship, native language and family relations. It also includes the date of emigration or death, if applicable. Data collection and registration is conducted on the basis of statutory notifications provided by national legislation and made by private individuals and public authorities.

The Population Information System is routinely used to define the eligible women to be invited for cervical cancer screening by their birth year. Data on women living in the committed municipalities in Southern Finland who belonged to the screening cohorts in years 2003–2007 were drawn from the Population Information System files. The same data source also provided sociodemographic information (I) as well as vital status and possible emigration needed to define person-years at risk (III, IV).

8.3.5. Use of the archived HPV DNA samples (IV)

After the screening visit the cervical exfoliated cells were stored in the standard transport medium (STM) at −20 ◦C for five to seven years. All the HC2-positive samples obtained during 2003–2005 were retrieved from the frozen archives and retrospectively genotyped.

From each tube 200 uL (STM) was treated overnight with proteinas K (20µg/mL, Roche) at 37ºC. Then DNA was extracted with the Total NA-kit (Roche) by the use of MagNA Pure LC (200 µL input and 100 µL output). Adequacy of the samples for the DNA extraction and HPV typing was assessed by testing five µL of the sample for the human ß-globin gene with a real time Polymerase Chain Reaction (PCR) (Sturegard et al. 2013). 5 µL of the extracted material was added to a total volume of 25 µL for modified general primer (MGP) PCR amplification.

In MGP, five forward and five reverse consensus primers have been modified from the classical GP5+/6+ primers to allow for an improved amplification of 14 oncogenic HPV types (Soderlund-Strand et al. 2009). The subsequent detection of MGP amplicons was done using the Luminex technology which allows for fast genotyping of all clinically relevant HPV types simultaneously. In the Luminex system small polystyrene beads, i.e. microspheres, are dyed with different amounts of two fluorophores and, thus, giving each bead a unique absorption spectra. Individual oligonucleotide probes for HPV types are coupled with the colour-coded beads which then can be identified with the reader system. For this study, the Luminex assay included probes for HPV types 6, 11, 16, 18, 26, 30, 31, 33, 35, 39, 40, 42, 43, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, 67, 68 (a and b), 69, 70, 73, 74, 81, 82, 83, 86, 87, 89, 90, 91 and 114 (Schmitt et al. 2006).

This extension of the randomised HPV screening trial was not considered routine screening practice. Concequently, an approval was obtained from The National Authority for Medicolegal Affairs to use previously taken cervical screening samples for research purposes. The extended register-based study was also judged ethically acceptable by the

ethical committee of the local hospital district. All personal identification information was concealed from the tubes and HPV samples were analysed in the Swedish laboratory (Medical Microbiology, Lund University, Malmö, Sweden) encrypted. Encrypted data containing HPV typing results was returned to the MSR to restore personal identifiers and an independent research register was comprised. This research register was then individually linked with the databases of the MSR, FCR and HDR.

8.3.6. Linkage of data sources (III and IV)

To obtain the best possible information on the cervical precancerous lesions and cancer detected in Finland, women were linked individually between three different health care registries using their personal identifiers. The diagnosis of invasive cervical cancer was based on data from the Finnish Cancer Registry only. Diagnosis of CIN 3/AIS was derived from the FCR in the first place and the information was completed from the MSR or the HDR in case that either database indicated diagnoses of interest.

CIN 1 and CIN 2 were available from MSR or HDR only, as these are not recorded in the FCR. Only one precancerous lesion per woman was allowed. We chose the most severe cervical lesion detected during the observation period, which was either 12 months after screening visit (IV) or a maximum of a full screening round of five years (III). In case that two or more registers indicated a cervical lesion of same stage, the earliest diagnosis was used.

The date of diagnosis in the HDR was the earliest date of admission that included the diagnosis of a cervical lesion of any stage. The date of diagnosis is routinely recorded in the FCR and in the MSR the date of the screening visit was used for this purpose.

8.4 Statistical analysis

8.4.1 HPV prevalence (I and IV)

Sociodemografic risk factors for cervical hrHPV infection were assessed using a binomial regression model and assuming multiplicative effects between explanatory variables estimates (Wacholder 1986). The GLM function was used for fitting the regression model and the actual fit of the model was, in turn, verified using Pearson's Chi Square Statistics (McCullagh and Nelder 1989).

The overall HPV prevalence was calculated as the proportion of HC2-positives women of all women tested for the presence of the HPV DNA. Type-specific proportion of HPV infection with exact binomial 95% confidence intervals (CI) were calculated for women overall and stratified by age and by cytologic and histologic findings. Type-specific HPV prevalence was calculated as the proportion of women positive for a given type, either

alone or with other concomitant types, in all HPV tested women (including HC2-negatives).

The associations between the HPV infection and a referral to a colposcopy as well as findings in histology were assessed by Poisson regression. We calculated relative rates with 95% CIs on a per protocol basis for HPV carcinogenicity categories and for HPV 16 individually. Women without a particular HPV type (i.e. women with other HPV infections or without any HPV type) were used as the referent group. RRs were adjusted for age and for other HPV types by including age as a continuous variable and type-specific HPV data as single variables in a multivariate regression model. Population attributable fractions (PAFs) were calculated from the formula:

adjPAF=p* (adjRR-1)/adjRR

where p is the proportion among cases and adjRR is the type-specific adjusted relative rate. 95% CIs for PAFs were calculated from the variance of the log-transformed complement of PAFs [log(1-PAF)] (Rothman et al. 2008).

8.4.2 Performance of HPV vs. cytology screening (II)

The performances of both the HPV DNA and the conventional cytology screening were assessed by test positivity rate, colposcopy referral rate, relative sensitivity measured through histologically confirmed detection rates, relative specificity and Positive Predictive Value. All the parameters of interest were assessed by random allocation. Performance parameters in the HPV DNA screening arm were estimated by Poisson regression using the conventional screening arm as the referent group. For the regression model, time at risk was specified as one for each subject (i.e. screening visit) (McNutt et al. 2003). Relative rate estimates were tested for effect modification by age including trial arm, age, and the trial arm–age interaction as covariates in the regression model.

Age-specific relative sensitivities were calculated from the number of screen-detected cervical lesions divided by the total number of women screened.

Age-specific Positive Predctive Values were calculated as the proportion of histologically confirmed cervical lesions of a given grade among women with a positive screening test result. Two different cut-offs for screening test positivity were used in the HPV DNA screening: 1) cytology triage test -positive (i.e. referral to a colposcopy), and 2) primary screening test (HC2) -positive. Age-specific RRs with their 95% CIs for test positivity at different cutoffs and for histological confirmation were estimated in the HPV DNA screening arm vs. conventional cytology arm.

Relative specificity estimates of the screening tests were calculated as the proportion of women with a negative screening test from all women with no histologically confirmed cervical lesion. Relative specificity approximates absolute specificity well when the prevalence of the disease, such as that of cervical cancer, is low. Two different cut-offs for test negativity were used in the HPV DNA screening: 1) cytology triage test -negative (i.e. no referral to colposcopy), and 2) primary screening test (HC2) -negative. Relative specificity between the two study arms were performed by modeling for age-specific false positivity rates (1 minus specificity).

8.4.3 Prospective detection rates in HPV vs. cytology screening (III)

The hazard (or detection) rates of CIN, AIS, or cervical cancer within one screening round of primary HPV screening were assessed by Poisson regression using cytology screening as the reference. Hazard ratios with 95% CIs were calculated for all women who were invited to a cervical screening. This included those who attended and also those who did not. Among the women who did attend screening, HRs were calculated also in three subgroups defined by womens´ screening status: 1) referral for colposcopy, 2) recommendation to an intensive screening and 3) negative or normal findings. All hazard ratio estimates were further stratified by age group at the time of randomisation (<35 years and ≥35 years). The association between the hazard (detection) of cervical lesion and age, and the potential effect modification between age and screening method were tested using likelihood ratio statistics.

For most of the women the follow-up did not reach the full screening round of five years. Therefore, also five year cumulative hazard (i.e. the cumulative detection rate) of CIN, AIS, or cervical cancer in both screening arms was assessed using the Nelson-Aalen estimator.

Statistical analyses were by random allocation for valid invitations throughout. For both the regression model and the Nelson-Aalen estimator, person-years at risk were used and they were calculated for every woman individually.

9 Results

9.1. Risk factors for cervical hrHPV infection (I)

During 2003─2004, 20 065 women were tested for the presence of HPV DNA by Hybrid Capture 2. Internal quality assurance checking indicated that two plates of HC2 test kit used for three months in the autumn of 2003 had probable defects in quality. These plates resulted in significantly frequent HC2+ results than plates used previously or after that. Therefore, 1 522 woman screened with these plates were excluded, leaving 16 895 women for the assessment of sociodemographic factors associated with an hrHPV infection.

The prevalence of a hrHPV infection ranged from 6.1% to 8.8% between municipalities. With regards to family relations, the prevalence of a hrHPV infection was 12.6% among unmarried women (including also women in a common-law marriage), 10.1% among divorced women whereas only 4.2% among married women. Nulliparous women had about a two fold hrHPV prevalence compared to women with at least one delivery (11.9% vs. 5–6%, respectively). Women who had undergone either a total or subtotal hysterectomy (n=1 682) had a lower hrHPV prevalence rate overall than women with no history of hysterectomy (5.4% vs. 7.8%, respectively). However, when adjusted for age, hysterectomised women had slightly higher hrHPV prevalences than non-hysterectomised women. Among women aged 35–49 years, 7.8% of those hysterectomised and 7.0% of those non-hysterectomised were hrHPV-positive. Among women aged 50–65 years old, corresponding estimates were 5.1% and 4.0%, respectively.

A multivariate regression analysis showed that age was a strong risk factor for an hrHPV infection as the RR decreased steadily with an increasing age (P for trend < 0.0001, Table 6). Crude RRs suggested that the risk of hrHPV infection increased with the size of the city and decreased with the number of births. However, the adjusted RRs showed no association between municipality or parity and the prevalence of hrHPV infection. Marital status was related to the hrHPV prevalence, married women being at the lowest risk for hrHPV infection. Multivariate regression analysis also suggested a total or subtotal hysterectomy being a risk factor for hrHPV infection (Table 6).

Table 6. *Multivariate analysis of risk factors for cervical hrHPV infection evaluated from the Finnish HPV screening trial in 2003-2004*

Variable	HC2 tested	HC2 positive		Crude RR	Adjusted RR (95 % CI)*
	n	n	%		
Age group (years)					
25−29	830	200	24.1	Ref.	Ref.
30−34	1 876	292	15.6	0.65	0.72 (0.61-0.86)
35−39	1 984	201	10.1	0.42	0.47 (0.39-0.58)
40−44	2 436	155	6.4	0.26	0.28 (0.23-0.36)
45−49	2 203	107	4.9	0.20	0.21 (0.17-0.27)
50−54	2 449	110	4.5	0.19	0.19 (0.15-0.24)
55−59	2 606	120	4.6	0.19	0.19 (0.15-0.24)
60−64	1 886	71	3.8	0.16	0.15 (0.11-0.20)
65+	625	18	2.9	0.12	0.11 (0.07-0.18)
					P for trend <0.0001
Municipality					
Helsinki	7 504	659	8.8	Ref.	Ref.
Vantaa	3 448	244	7.1	0.81	1.05 (0.88-1.26)
Espoo	2 330	141	6.1	0.69	1.05 (0.71-1.55)
Other	3 613	230	6.4	0.72	1.07 (0.78-1.48)
Marital status					
Married/Widowed	9 211	382	4.2	Ref.	Ref.
Unmarried	4 823	606	12.6	3.03	1.78 (1.53-2.08)
Divorced/Separated	2 841	286	10.1	2.43	2.98 (2.57-3.46)
Parity					
0†	4 704	558	11.9	Ref.	Ref.
1	3 487	214	6.1	0.52	0.87 (0.74-1.03)
2	5 850	342	5.6	0.49	1.06 (0.90-1.25)
3	2 220	130	5.9	0.49	1.11 (0.90-1.37)
≥4	634	30	4.7	0.40	0.89 (0.61-1.30)
					P for trend <0.400
Hysterectomy					
Not done	15 213	1 184	7.8	Ref.	Ref.
Done	1 682	90	5.4	0.69	1.37 (1.09-1.72)

* Adjusted for all the other explanatory variables in the regression model.

† Including women for which no information on parity was recorded. Adapted from the Leinonen M, Kotaniemi-Talonen L, Anttila A, Dyba T, Tarkkanen J, Nieminen P. Prevalence of oncogenic human papillomavirus infection in an organised screening population in Finland. Int J Cancer. 123(6):1344-9. Copyright (2008) by permission of Wiley-Liss, Inc.

9.2. Age-specific prevalence of HPV infection (I and IV)

33 043 women were screened by the HC2 test during 2003–2005. The mean age of the HC2 tested women was 45.2 years (range 25–65). 11 samples that were initially HC2-positive were not retrospectively identified from the archives and 2 611 samples were targeted for genotyping. 80 tubes (3.1 %) did not contain a measurable amount of human β-globin DNA but 43 of them contained viral DNA. 37 samples contained neither β-globin nor HPV DNA and they were excluded.

Overall, 2 574 women (7.8% of tested) were positive by the HC2 test and underwent triage testing by cytology and genotyping by a PCR. 773 (30.0%) of the HC2-positive samples were found to be negative by the PCR. Among women aged 25–29 years, the proportion of PCR-/HC2+ -results was 25% whereas among women aged ≥ 65 years it was over 50%. Following the highest value within the age bracket of 25–29 years, a rather constant decline in hrHPV prevalence was seen over ages by both test methods. Among women who were of the screening target ages (those aged 30–60 years), hrHPV prevalence ranged from 5% to 15% using the HC2 test and from 2% to 10% using the PCR (Figure 7).

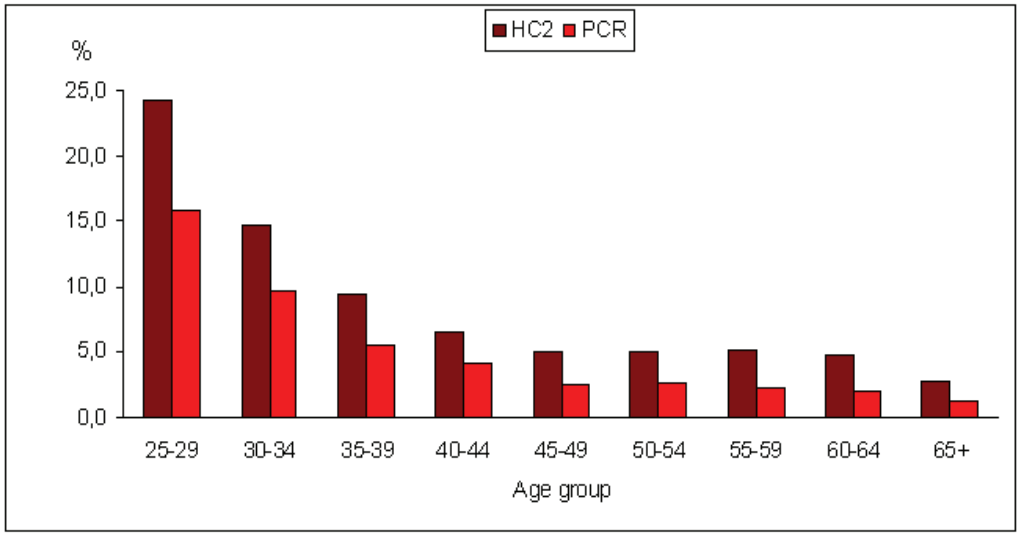


Figure 7 *Age-specific prevalence of the hrHPV infection in screening population by HPV test method in the Finnish HPV screening trial at the index screen during 2003–2005*

9.3. Type-specific proportion of HPV infection (IV)

Of the 2 574 initially HC2-positive samples, 57.1% contained carcinogenic HPV DNA (Group 1/2A) using the PCR. 15.3% of the samples contained possibly carcinogenic Group 2B types and 8.5% Group 3 low-risk types which are not targeted by the HC2 test. Group 2B HPV type was detected as the most severe infection in 10.3% of the samples and Group 3 HPV type in 2.6% of them, respectively. Among HC2-positive women, infection with Group 1/2A HPV types decreased from the highest value of 65.6% among women younger than 35 years to 41.2% among women aged ≥ 65 years whereas Group 2B and Group 3 HPV types were slightly more often detected at older age groups (Figure 8).

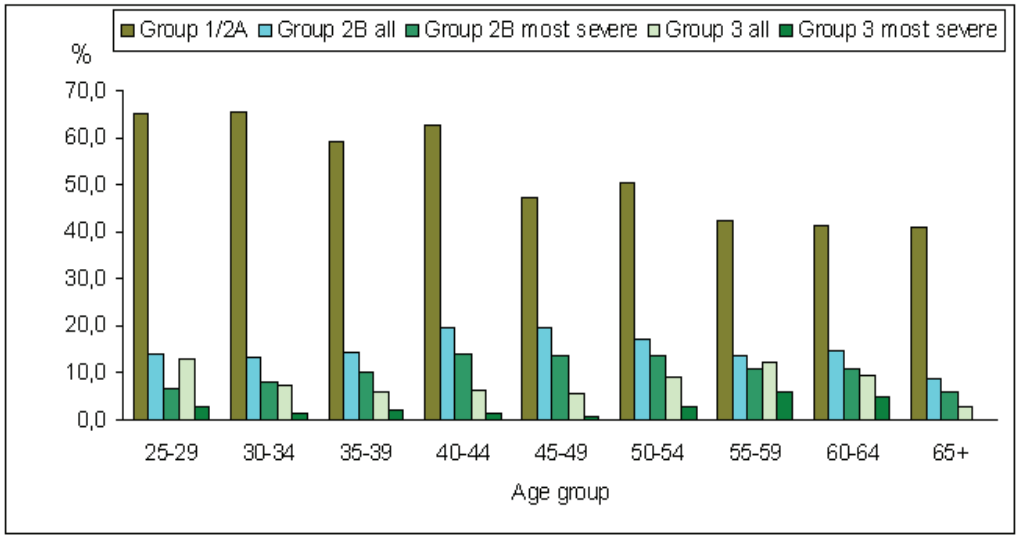


Figure 8 *Age-specific results of PCR amplification by HPV carcinogenicity categories in women with positive Hybrid Capture 2 test result in the Finnish HPV trial during 2003–2005*

Based on the classification by the International Agency for Research on Cancer (IARC).
Group 1/2A: at least one type (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), coinfection with Group 2B and / or Group 3 types allowed.
Group 2B all: at least one type (26, 30, 53, 66, 67, 69, 70, 73 and 82), coinfection with Group 1/2A and / or Group 3 types allowed.
Group 2B most severe: coinfection only with Group 3 types allowed.
Group 3 all: infection with one or more type (6, 7, 9, 11, 40, 42, 43, 61, 74, 81, 83, 86, 87, 89, 90, 91 and 114), coinfection with Group 1/2A and / or Group 2B types allowed.
Group 3 most severe: coinfection with types of other groups not allowed.

Table 7. *Type- and age-specific proportion of hrHPV types among women screened by a primary HC2 test in the Finnish HPV screening trial during 2003–2005*

	Prevalence (%)										
	Age group (years)										
	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65+	among all (95% CI)	among HC2+ (95% CI)
HPV 16	4.4	2.5	1.1	0.8	0.3	0.4	0.4	0.4	-	0.9 (0.8-1.1)	12.2 (10.9-13.5)
HPV 18	1.8	0.7	0.5	0.2	0.2	0.1	0.1	0.2	0.2	0.4 (0.3-0.4)	4.5 (3.8-5.4)
HPV 31	1.8	2.0	1.2	0.5	0.3	0.3	0.3	0.3	0.3	0.7 (0.6-0.8)	8.9 (7.9-10.1)
HPV 33	1.4	0.9	0.3	0.2	0.2	0.2	0.1	0.1	-	0.3 (0.3-0.4)	4.0 (3.3-4.9)
HPV 35	0.3	0.3	0.2	0.1	0.05	0.2	0.08	0.1	-	0.2 (0.1-0.2)	2.1 (1.5-2.7)
HPV 39	1.1	0.4	0.3	0.2	0.2	0.2	0.1	0.1	-	0.3 (0.2-0.3)	3.3 (2.6-4.1)
HPV 45	1.0	0.6	0.5	0.4	0.3	0.2	0.2	0.1	0.2	0.3 (0.3-0.4)	4.4 (3.6-5.3)
HPV 51	1.9	0.7	0.4	0.3	0.09	0.2	0.2	0.2	-	0.4 (0.3-0.4)	4.6 (3.8-5.5)
HPV 52	1.7	0.9	0.6	0.5	0.4	0.3	0.3	0.2	0.2	0.5 (0.4-0.6)	6.3 (5.4-7.3)
HPV 56	1.4	0.7	0.3	0.3	0.2	0.2	0.2	0.2	-	0.3 (0.3-0.4)	4.2 (3.4-5.0)
HPV 58	2.2	0.8	0.4	0.3	0.1	0.2	0.2	0.2	0.2	0.4 (0.3-0.4)	5.0 (4.2-5.9)
HPV 59	0.7	0.2	0.2	0.2	0.2	0.04	0.08	0.03	-	0.2 (0.1-0.2)	1.9 (1.4-2.6)
HPV 68	1.0	0.5	0.3	0.3	0.2	0.3	0.2	0.3	0.2	0.3 (0.3-0.4)	4.3 (3.5-5.1)

Type-specific prevalence was calculated as a proportion of women positive for a given type out of all HPV tested women including HC2-negatives.
Type-specific prevalence among HC2-positive samples, partly previously unpublished data.

The most common HPV type among women screened by the HC2 test during 2003–2005 was HPV 16, followed by HPV 31 and HPV 52. HPV 16 together with other types of α9 species accounted for 38.4% of HC2-positive samples and 54.9% of samples with a positive result in genotyping by the PCR. Most of the Group 1 HPV types contributed from 4.0% to 5.0% of HC2-positive samples except for HPV types of 35, 39 and 59 which were less frequently detected. The type- and age-specific proportion of hrHPV types among HC2 tested women and among HC2-positive samples are given in Table 7.

9.6% of HC2-positive women with LSIL or worse cytology were negative by PCR-based genotyping. The respective proportion among women with ASCUS was 22.9% and among women with normal cytology 37.9%. The proportion of Group 1/2A HPV types increased with severity of cytology. Their prevalence was about 50% when cytology was classified as normal and almost 80% when it indicated a referral to a colposcopy. Group 2B HPV type as the most severe infection was most frequently detected with the ASCUS cytology. The proportion of only Group 3 HPV types was 3.4% (95% CI 2.5-4.4) when there were no signs of cellular atypia and 1.3% (0.7-2.2) when cytology indicated ASCUS or worse. In general, type-specific proportion of hrHPV types slightly differed among HC2-positive women with normal and ASCUS cytology. HPV 16 and related types of α9 species were detected most often among women with LSIL+ cytology except for HPV 52 and HPV 58 which were more frequent among women with ASCUS (Table 8).

Any cervical lesions or cancer was diagnosed in 54.5% of women with LSIL+ cytology and overall in 0.7% of women who attended screening. A total of 238 cervical lesions included 21 CIN 1+ lesions diagnosed within 12 months following a screening visit after ASCUS or a normal result in cytology triage. The proportion of Group 1/2A HPV types increased with increasing CIN grade and over 90% of women with CIN 3+ lesion were positive for the HPV DNA of Group 1/2A types. When more severe HPV types were excluded, infections within the Group 2B types were overrepresented in women with CIN 1 (Table 8). Principally, there was no difference in type-specific proportion among women with CIN 1 lesion or negative result in colposcopy (data not shown). The proportion of HPV 16 and HPV 52 increased whereas that of HPV 51 decreased with an increasing CIN grade. A total of three cancers were detected of which two were adenocarcinomas and one was a minimally invasive squamous cell carcinoma (formerly microinvasive). The types associated with invasive cancer were HPV 16 in two cases and HPV 45 in one case.

Group 1/2A HPV types attributed to 78.3% (95% CI 53.4-89.9) of CIN 3+ cases. HPV 16 alone attributed to 55.8% (95% CI 40.0-67.5) of CIN 3+ cases and 19.5% (14.4-24.3) of colposcopy referrals. Group 2B HPV types attributed to 5.9% of colposcopy referrals (95% CI 1.5-10.1) but were not associated with high-grade cervical lesions, and Group 3 HPV types did not cause any burden on colposcopies or on cervical diagnosis (data not shown).

Table 8. *Type-specific proportion of the HPV infection with exact binomial 95 % confidence intervals (CI) in the Finnish HPV screening trial during 2003–2005 by cytologic and histologic finding at the index screen*

Type(s)	Cytology triage						Histologic diagnosis					
	Normal (n=1582)		ASCUS (n=594)		LSIL+ (n=398)		CIN 1 (n=71)		CIN 2 (n=104)		CIN 3+ (n=63)	
	n	% (95 % CI)	n	% (95 % CI)	n	% (95 % CI)	n	% (95 % CI)	n	% (95 % CI)	n	% (95 % CI)
HPV 16	143	9.0 (7.7-10.6)	58	9.8 (7.5-12.4)	112	28.1 (23.8-32.8)	11	15.5 (8.0-26.0)	40	38.5 (29.1-48.5)	38	60.3 (47.2-72.4)
HPV 18	64	4.0 (3.1-5.1)	29	4.9 (3.3-6.9)	24	6.0 (3.9-8.8)	4	5.6 (1.6-13.8)	6	5.8 (2.1-12.1)	2	3.2 (0.4-11.0)
HPV 31	117	7.4 (6.2-8.8)	60	10.1 (7.8-12.8)	53	13.3 (10.1-17.1)	4	5.6 (1.6-13.8)	24	23.1 (15.4-32.4)	8	12.7 (5.6-23.5)
HPV 33	52	3.3 (2.5-4.3)	23	3.9 (2.5-5.8)	29	7.3 (4.9-10.3)	5	7.0 (2.3-15.7)	6	5.8 (2.1-12.1)	5	7.9 (2.6-17.6)
HPV 35	24	1.5 (1.0-2.2)	18	3.0 (1.8-4.7)	11	2.8 (1.4-4.9)	2	2.8 (0.3-9.8)	4	3.8 (2.1-12.1)	1	1.6 (0.04-8.5)
HPV 39	45	2.8 (2.1-3.8)	21	3.5 (2.2-5.4)	19	4.8 (2.9-7.4)	3	4.2 (0.9-11.9)	4	3.8 (2.1-12.1)	1	1.6 (0.04-8.5)
HPV 45	68	4.3 (3.4-5.4)	30	5.1 (3.4-7.1)	15	3.8 (2.1-6.1)	3	4.2 (0.9-11.9)	3	2.9 (0.6-8.2)	1	1.6 (0.04-8.5)
HPV 51	51	3.2 (2.4-4.2)	31	5.2 (3.6-7.3)	36	9.0 (6.4-12.3)	13	18.3 (10.1-29.3)	4	3.8 (1.1-9.6)	1	1.6 (0.04-8.5)
HPV 52	96	6.1 (4.9-7.4)	44	7.4 (5.4-9.8)	21	5.3 (3.3-8.0)	1	1.4 (0.04-7.6)	7	6.7 (2.7-13.4)	5	7.9 (2.6-17.6)
HPV 56	49	3.1 (2.3-4.1)	31	5.2 (3.6-7.3)	27	6.8 (4.5-9.7)	5	7.0 (2.3-15.7)	2	1.9 (0.2-6.8)	2	3.2 (0.4-11.0)
HPV 58	66	4.1 (3.2-5.2)	41	6.9 (5.0-9.2)	21	5.3 (3.3-8.0)	4	5.6 (1.6-13.8)	7	6.7 (2.7-13.4)	3	4.8 (1.0-13.3)
Group 1/2A*	777	49.1 (46.6-51.6)	376	63.3 (59.3-67.2)	318	79.9 (75.6-83.7)	51	71.8 (59.9-81.9)	88	84.6 (76.2-90.9)	57	90.5 (80.4-96.4)
Group 2B†	153	9.7 (8.3-11.2)	74	12.5 (9.9-15.4)	37	9.3 (6.6-12.6)	13	18.3 (10.1-29.3)	4	3.8 (1.1-9.6)	1	1.6 (0.04-8.5)
Any HPV	983	62.1 (59.7-64.5)	458	77.1 (73.5-80.4)	360	90.5 (87.1-93.2)	65	91.5 (82.5-96.8)	92	88.5 (80.7-93.9)	58	92.1 (82.4-97.4)

* Group 1/2A: at least one type (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), coinfection with Group 2B and / or Group 3 types allowed

† Group 2B: at least one type (26, 30, 53, 66, 67, 69, 70, 73 and 82), coinfection with Group 3 types allowed

9.4 Age-specific distribution of cytology (II and IV)

Altogether 108 425 randomised invitations were sent out as part of the Finnish HPV screening trial between the years 2003 and 2005. 54 207 invitations were allocated to primary HPV DNA screening, for which 35 837 (66.1%) women attended. From the remaining 54 218 invitations for the conventional screening arm, 35 500 (65.5%) attended the screening. In the HPV arm, 33 100 women were screened with a primary HC2 test (92.4%) and 2 737 women with a primary cytology (7.6%). 2 628 women (7.3% of screened) were HC2-positive and 2 626 were triaged with cytology. Cytology was analysed also for 3 436 HC2-negative women as they reported abnormal bleeding symptoms (data not shown).

In the conventional arm, 35 475 women (99.9%) were screened according to the protocol by the cytology. In addition, four cytology triage tests were performed for women screened erroneously by the primary HC2 test. The cytological results were as follows: 1.2% of women had LSIL or worse, 7.0% had ASCUS (including reactive changes) or worse, and 92.7% had normal cytology (Figure 9).

In the HPV arm, cytology was read for 2 626 women as a triage test and for 2 737 women as a primary screening test. Of the 5 363 cytological smears taken, 7.9% were classified as LSIL or worse, 21.8% were ASCUS or worse, and 77.9% showed no signs of cellular atypia (Figure 10). The number of unsatisfactory Pap smears was very low in both screening arms (16 in the HPV vs. 79 in the conventional arm). Cytology triage results from the study IV including only HC2-positive samples (n= 2 574) showed slightly higher rates of abnormal smears: 15.5% of HC2-positive women had LSIL+ cytology, 23.1% had ASCUS and 61.5% had a normal smear.

9.5. Performance of HPV vs. cytology screening (II)

The mean age of women who attended a screening in the HPV arm (n= 35 837) between 2003 and 2005 was 45.2 years. The corresponding age for that of the conventional arm (n= 35 500) was 45.3 years. The women who attended a screening were also similar in terms of their municipality, screening laboratory, marital status, and parity (data not shown).

At the index screen visit, 2 581 women were recommended to intensive screening in the HPV screening arm and 2 340 in the conventional arm (7.2% vs. 6.6% of the screened). The intensive screening was most often indicated among women 25- to 29-year olds, the rate being 21.9% in the HPV arm vs. 10.0% in the conventional arm (Figure 11).

Recommendations for intensive screening decreased with increasing age in both arms (P-value for age and P-value for a trend < 0.001). From the age of 40 years onwards, the rate was constantly lower in the HPV arm than that in the conventional arm. Overall, there were 9% more recommendations for intensive screening in the HPV arm in comparison with the conventional arm (95% CI 3-15%). However, the number of women aged from 25 to 34 years who were recommended to participate in intensive screening was substantially higher in the HPV arm than in the conventional arm (RR 2.20; 95% CI 1.96-2.47). This was mainly due to a large number of HC2-positive women with a normal result

in cytology triage. In both arms, the proportion of women who were recommended to participate in intensive screening decreased with increasing age. From the age of 40 years onwards, the rate of recommendations for intensive screening was constantly lower in the HPV arm compared with the conventional arm.

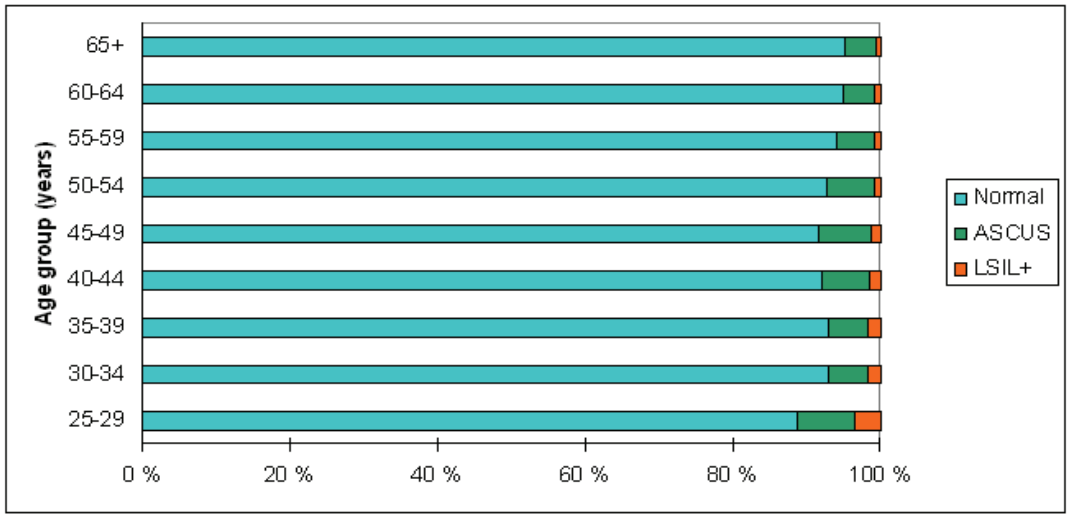


Figure 9 *Age-specific distribution of cytology in the conventional screening arm in the Finnish HPV sceening trial at the index screen in 2003–2005*

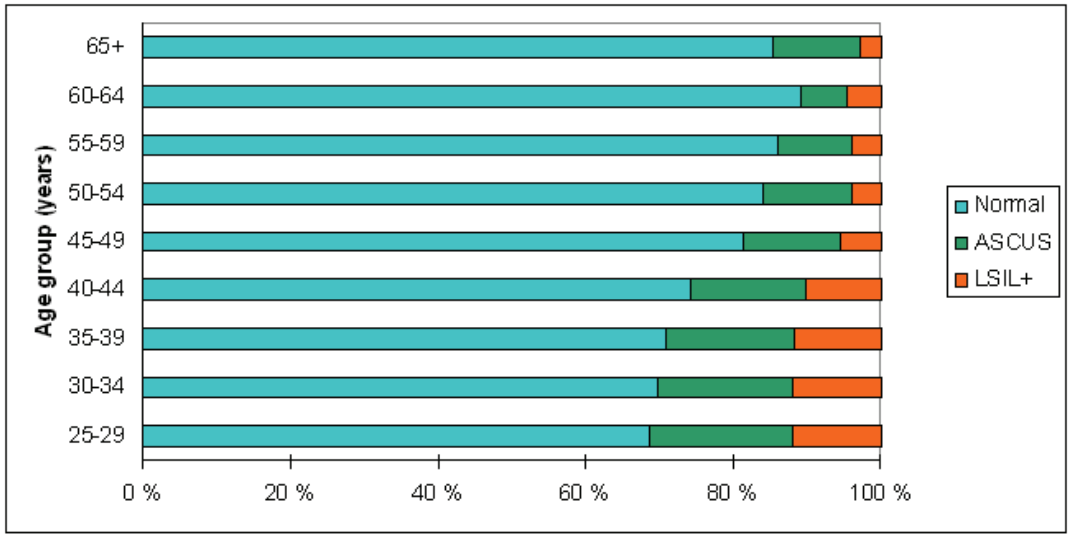


Figure 10 *Age-specific distribution of cytology in the HPV screening arm including primary cytology and triage tests done in the Finnish HPVscreening trial at the index screen in 2003–2005*

Overall, there was no difference in referrals to colposcopy between the two screening methods at the index screen. 1.2% of the women were recalled in both arms (RR 1.00; 95% CI 0.87-1.14). Among women under 35 years of age, the RR of colposcopy referral was 1.27 (95% CI 1.01-1.60) in the HPV arm vs. in the conventional arm. As with recommendations for intensive screening, the colposcopy referrals varied somewhat across the different age groups (P-value for age < 0.001) but no systematic pattern was seen.

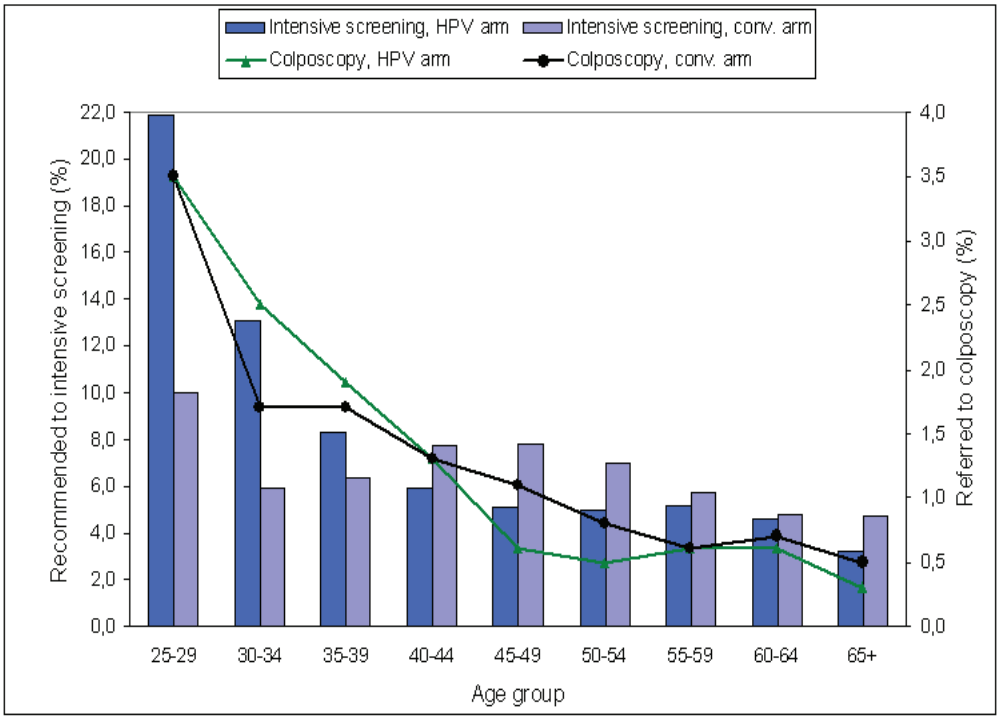


Figure 11 *Age-specific proportion of colposcopy referrals and recommendations to intensive screening done in the Finnish HPV trial in 2003–2005 at the index screen by screening method. Conv: conventional*

A histologically confirmed cervical precancerous lesion (CIN, AIS) or cancer was detected in 0.59% of the women invited for screening in the HPV screening arm and in 0.43% of the women in the conventional arm. CIN 2 lesions were the most frequently detected ones in both arms. The HPV screening method resulted in an increase of about a third in the detection of any cervical lesions in comparison with the conventional cytology screening (RR 1.37; 95% CI 1.11-1.69). The age-specific detection rates of cervical precancerous lesions and cancer are given in Table 9. The relative rates of detection varied significantly over the different age groups (*P* for age < 0.001 for all histological outcomes) but not a clear trend was observed.

Table 9. *Age-specific results of histologically confirmed cervical lesions in the HPV vs. conventional screening arm detected in the Finnish HPV screening trial at the index screen during 2003–2005*

Age	Histology											
	CIN 1			CIN 2			CIN 3+			Any CIN or cancer		
	n		RR (95% CI)	n		RR (95% CI)	n(*)		RR (95% CI)	n		RR (95% CI)
	HPV screening	Conv. screening		HPV screening	Conv. screening		HPV screening	Conv. screening		HPV screening	Conv. screening	
25–34	24	16	1.46 (0.78–2.75)	51	35	1.42 (0.92–2.18)	10 (1)	11 (1)	0.88 (0.38–2.08)	85	62	1.33 (0.96–1.85)
35–44	31	16	1.93 (1.05–3.53)	36	24	1.49 (0.89–2.50)	19 (1)	10 (1)	1.89 (0.88–4.07)	86	50	1.71 (1.21–2.43)
45–54	6	12	0.49 (0.18–1.31)	7	11	0.62 (0.24–1.61)	7 (2)	7 (2)	0.98 (0.34–2.80)	20	30	0.65 (0.37–1.15)
≥ 55	6	2	3.01 (0.61–14.9)	10	4	2.51 (0.79–8.00)	6 (2)	6	1.00 (0.32–3.11)	22	12	1.84 (0.91–3.72)
Total	67	46	1.44 (0.99–2.10)	104	74	1.39 (1.03–1.88)	42	34	1.22 (0.78–1.92)	213	154	1.37 (1.11–1.69)
	P for age < 0.001 *P* for trend = 0.76			*P* for age < 0.001 *P* for trend = 0.90			*P* for age < 0.001 *P* for trend = 1.00			*P* for age < 0.001 *P* for trend = 0.86		

* Number of invasive cancers from the total of CIN 3 or worse lesions within brackets. Conv. = Conventional

In terms of all histological outcomes, the specificity of the HC2 test with cytology triage was equal to that of cytology screening whereas the specificity of the HC2 test alone was clearly inferior. For instance, the specificity of the HC2 test with cytology triage for any cervical precancerous lesion or cancer (CIN 1+) was 99.4% and that of cytology was 99.2%, whereas the specificity of the HC2 test alone was only 93.2%. Considering all age groups, the specificity of the HC2 test with cytology triage was statistically significantly better than that of conventional cytology for CIN 1+ ($p = 0.0091$). For more severe histological outcomes of CIN 2+ and CIN 3+, there was no difference in specificity between the two screening methods (Tables 10 and 11).

The specificity of the screening method was dependent on the woman´s age and on the grade of the histological lesion in both screening arms. Regardless of the screening method or the histological outcome of interest, the specificity of the screening method increased with the age of the woman and decreased with an increasing CIN grade. HPV screening with cytology triage tended to have a better specificity for all lesions among women 35 years and older than the conventional cytology. Also the specificity of the HC2 test alone increased with the age of the woman but was in all age groups significantly lower than that of HPV screening with cytology triage or that of conventional cytology (Tables 10 and 11).

In comparison with cytology, PPVs for HPV testing with cytology triage were consistently higher for all studied histological outcomes. The relative PPV in the HPV arm for CIN 1+ was 1.37 (95% CI 1.11-1.69), for CIN 2+ 1.34 (1.04-1.72) and for CIN 3+ 1.22 (0.78-1.92) compared to the conventional arm. The PPVs of the HC2 test alone were only about one fifth compared to those of conventional cytology for every histological outcome. The PPV of the HC2 test alone was lowest at 1.6% for CIN 3+ and highest at 8.1% for CIN 1+. In both screening arms, the highest PPVs were observed among women <35 years. However, the association between PPV and age decreased with an increasing CIN grade (Tables 10 and 11).

9.6. Prospective detection rates in HPV vs. cytology screening (III)

There were 203 788 women eligible for randomisation between years 2003 and 2007 of which 363 (0.2%) subjects were excluded due to the end of the follow-up period before the 1st of January each screening year (Figure 12, see material and methods). Thus, there were 101 678 valid invitations in the HPV screening arm and 101 747 in the conventional screening arm. Of the invited women, 66 410 (65.3%) attended screening in the HPV arm and 65 785 (64.7%) in the conventional arm. 724 891 person years at risk for invasive cervical cancer and 720 937 person years at risk for cervical precancerous lesion accumulated during an average observation period of 3.6 years (standard deviation 1.2, range from 0 to 5 years). The person years at risk by study arm, attendace and index screening status are given in Table 12.

Table 10. *Age-specific performance of screening for CIN 1+ and CIN 2+ lesions in HPV vs. conventional screening arm in the Finnish HPV screening trial at the index screen in 2003–2005. In the HPV arm two definitions for screening test positivity/negativity were used: referral to a colposcopy (cytology triage positive/negative) and a primary test positive/negative*

Age group (years)	Test positive	Test negative	CIN 1+				CIN 2+			
	n	n	Specificity (%)	RR of false positive (95% CI)	PPV (%)	Relative PPV (95 % CI)	Specificity (%)	RR of false positive (95% CI)	PPV (%)	Relative PPV (95 % CI)
HPV, cytology triage										
25–34	166	5 703	98.6	1.22 (0.88–1.69)	51.2	1.05 (0.76–1.46)	98.2	1.26 (0.95–1.69)	36.7	1.01 (0.69–1.49)
35–44	143	9 049	99.4	0.66 (0.47–0.93)	60.1	1.64 (1.15–2.32)	99.0	0.86 (0.65–1.14)	38.5	1.54 (1.00–2.36)
45–54	54	9 584	99.6	0.57 (0.38–0.83)	37.0	1.09 (0.62–1.91)	99.6	0.56 (0.38–0.83)	25.9	1.27 (0.63–2.55)
55–65	61	11 077	99.6	0.69 (0.46–1.03)	37.7	2.07 (1.03–4.19)	99.6	0.77 (0.52–1.13)	26.2	1.81 (0.82–3.99)
Total	424	35 413	99.4	0.79 (0.66–0.94)	50.1	1.37 (1.11–1.69)	99.2	0.88 (0.75–1.04)	34.4	1.34 (1.04–1.72)
				P for age < 0.001		*P* for age < 0.001		*P* for age < 0.001		*P* for age = 0.005
				P for trend = 0.01		*P* for trend = 0.14		*P* for trend = 0.06		*P* for trend = 0.17
HPV, primary test										
25–34	983	4 880	84.4	13.6 (10.6–17.5)	8.6	0.17 (0.12–0.24)	84.0	11.2 (8.90–14.0)	6.2	0.17 (0.11–0.24)
35–44	681	8 506	93.4	6.97 (5.56–8.74)	12.6	0.34 (0.24–0.48)	93.1	6.17 (5.01–7.61)	8.1	0.31 (0.20–0.48)
45–54	464	9 167	95.3	7.62 (5.80–10.0)	4.3	0.13 (0.07–0.22)	95.3	6.40 (4.98–8.23)	3.0	0.15 (0.07–0.30)
55–65	500	10 632	95.6	8.54 (6.49–11.2)	4.6	0.23 (0.11–0.47)	95.6	8.35 (6.37–10.9)	3.2	0.19 (0.08–0.43)
Total	2 628	33 185	93.2	9.10 (8.02–10.3)	8.1	0.21 (0.17–0.26)	93.0	7.96 (7.08–8.96)	5.6	0.21 (0.16–0.27)
				P for age < 0.001		*P* for age < 0.001		*P* for age < 0.001		*P* for age < 0.001
				P for trend = 0.01		*P* for trend = 0.76		*P* for trend = 0.06		*P* for trend = 0.73
Conventional arm										
25–34	127	5 584	98.6	Ref.	48.8	Ref.	98.6	Ref.	36.2	Ref.
35–44	136	9 012	98.9		36.8		98.9		25.0	
45–54	88	9 366	99.3		34.1		99.3		20.5	
55–65	69	11 118	99.5		17.4		99.5		14.5	
Total	420	35 080	99.1		36.7		99.1		25.7	

Table 11. *Age-specific performance of screening for CIN 3+ lesions in HPV vs. conventional screening arm in the Finnish HPV screening trial at the index screen in 2003–2005. In the HPV arm two definitions for screening test positivity and negativity were used: referral to a colposcopy (cytology triage positive/negative) and a primary screening test positive/negative*

Age group (years)	Test positive	Test negative	CIN 3+					
	n	n	Specificity (%)	RR of false positive	95% CI	PPV (%)	Relative PPV	95 % CI
HPV arm with cytology triage								
25–34	166	5 703	97.3	1.31	1.03–1.66	6.0	0.70	0.30–1.64
35–44	143	9 049	98.6	0.98	0.77–1.26	13.3	1.81	0.84–3.89
45–54	54	9 584	99.5	0.57	0.40–0.82	13.0	1.63	0.57–4.65
55–65	61	11 077	99.5	0.88	0.61–1.26	9.8	1.13	0.36–3.51
Total	424	35 413	98.9	0.98	0.85–1.13	9.9	1.22	0.78–1.92
p-value for age					$p < 0.001$			$p = 0.20$
p-value for a trend					$p = 0.004$			$p = 0.44$
HPV arm with primary test								
25–34	983	4 880	83.3	8.21	6.77–9.95	1.0	0.12	0.05–0.28
35–44	681	8 506	92.7	5.27	4.36–6.38	2.8	0.36	0.17–0.78
45–54	464	9 167	95.2	5.62	4.44–7.11	1.5	0.19	0.07–0.54
55–65	500	10 632	95.5	7.97	6.13–10.4	1.2	0.12	0.04–0.38
Total	2 628	33 185	92.7	6.70	6.02–7.46	1.6	0.19	0.12–0.30
p-value for age					$p < 0.001$			$p = 0.71$
p-value for a trend					$p = 0.60$			$p = 0.95$
Conventional arm								
25–34	127	5 584	98.0	Ref.		8.7	Ref.	
35–44	136	9 012	98.6			7.4		
45–54	88	9 366	99.1			8.0		
55–65	69	11 118	99.4			8.7		
Total	420	35 080	98.9			6.0		

There were an equal number of referrals to colposcopy at the index screen in the HPV and in the conventional arm (1.2% vs. 1.1% among attenders, respectively). 6.7% of women who attended screening were recommended to participate in intensive screening after the primary HC2 test and 5.7% after the primary cytology. A slightly fewer women were test negative at the index screen in the HPV than in the conventional arm (92.1% vs. 93.1%), see Figure 12 and Table 12.

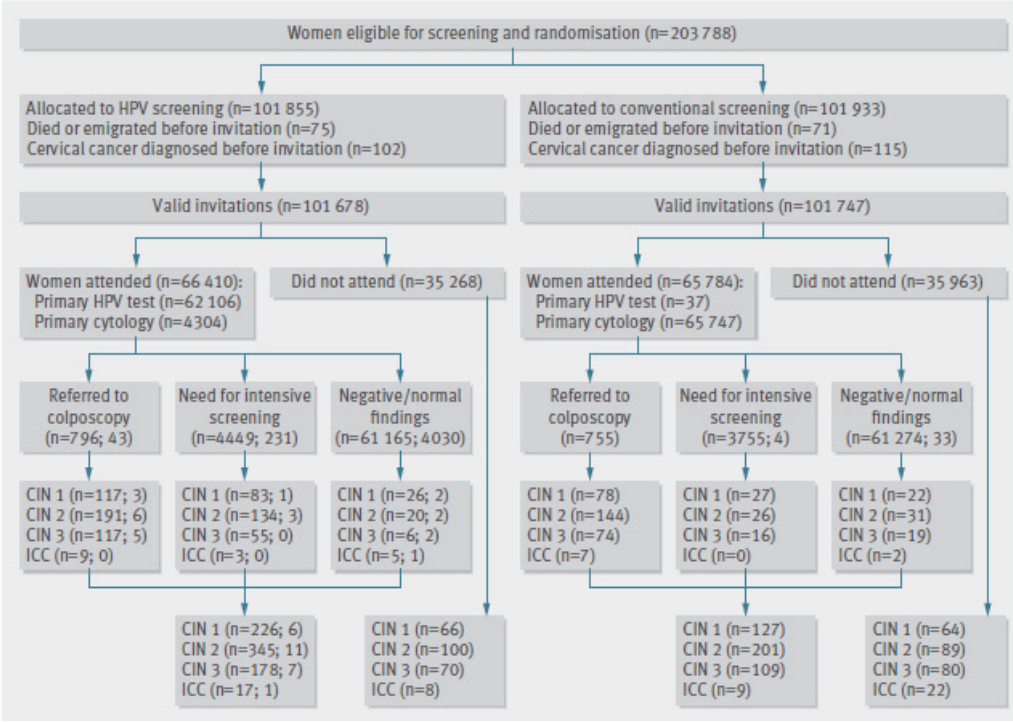


Figure 12 *Flowchart of the screening profiles of women invited for cervical screening in the Finnish HPV screening trial in 2003-2007. Data after semicolons represent the number of women who were not screened according to their random allocation. Reprinted from Leinonen et al. BMJ 2012;345:e7789.*

There were altogether 1 010 cases of cervical precancerous lesions or cancer among women invited for screening in the HPV screening arm and 701 in the conventional arm. The number of CIN 1+ lesions among attenders was 766 and 446, respectively. All cervical precancerous lesions and cancers detected during follow-up by study arm, age and participation as well as by screening status at the index screen visit are given in Figure 12 and in Table 13.

Table 12. *Person years at risk and proportions of cervical lesions by screening arm, age at randomisation and status at index screen among women invited for cervical screening in the Finnish HPV screening trial in 2003–2007*

	HPV screening			Conventional screening		
		Person years at risk (%)			Person years at risk (%)	
Participant status	No of women	Invasive cervical cancer	Cervical lesion of any grade	No of women	Invasive cervical cancer	Cervical lesion of any grade
All invited	101 678	362 318 (100.0)	360 017 (100.0)	101 747	362 573 (100.0)	360 919 (100.0)
25–34 years	20 460	71 512	70 553	20 455	71 548	70 882
≥ 35 years	81 218	290 806	289 464	81 292	291 025	290 037
Attenders	66 410	238 714 (65.9)	236 946 (65.8)	65 784	236 548 (65.2)	235 431 (65.2)
25–34 years	11 191	39 620	38 913	11 071	38 942	38 535
≥ 35 years	55 219	199 094	198 033	54 713	197 606	196 896
Non-attenders	35 268	123 603 (34.1)	123 071 (34.2)	35 963	126 025 (34.8)	125 488 (34.8)
25–34 years	9269	31 891	31 641	9384	32 606	32 347
≥ 35 years	25 999	91 712	91 430	26 579	93 419	93 141
Colposcopy referral	796	2842 (0.8)	1559 (0.4)	755	2670 (0.7)	1787 (0.5)
25–34 years	290	1066	548	211	767	443
≥ 35 years	506	1776	1011	544	1903	1344
Intensive screening	4449	15 909 (4.4)	15 510 (4.3)	3755	13 515 (3.7)	13 400 (3.7)
25–34 years	1686	5889	5724	669	2323	2297
≥ 35 years	2763	10 020	9787	3086	11 192	11 103
Negative or normal findings	61 165	219 963 (60.7)	219 876 (61.1)	61 274	220 362 (60.8)	220 244 (61.0)
25–34 years	9215	32 665	32 641	10 191	35 852	35 795
≥ 35 years	51 950	187 298	187 236	51 083	184 510	184 449

Adapted from Leinonen et al. BMJ 2012;345:e7789.

Table 13. *Hazard ratios of cervical precancerous lesion or cancer among women invited for cervical screening in the Finnish HPV screening trial during 2003–2007 by participation and screening statuses*

	No of cases Age 25–34 years		No of cases Age ≥ 35 years		Hazard ratio (95% CI)		
	HPV screening	Conventional screening	HPV screening	Conventional screening	Age 25–34 yrs	Age ≥ 35 yrs	Overall
All invited							
ICC	1	6	24	25	0.17 (0.02–1.39)	0.96 (0.55–1.68)	0.81 (0.48–1.37)
CIN 3 or AIS	90	70	158	119	1.29 (0.95–1.77)	1.33 (1.05–1.69)	1.32 (1.09–1.59) ***
CIN 2	209	135	236	155	1.56 (1.25–1.93)	1.53 (1.25–1.87)	1.54 (1.33–1.78) ***
CIN 1	117	68	175	123	1.73 (1.28–2.33)	1.43 (1.13–1.80)	1.53 (1.28–1.84) ***††
Attenders							
ICC	1	2	16	7	0.49 (0.04–5.42)	2.27 (0.93–5.51)	1.87 (0.83–4.20)
CIN 3 or AIS	62	32	116	77	1.92 (1.25–2.94)	1.50 (1.12–2.00)	1.62 (1.28–2.06) ***
CIN 2	155	85	190	116	1.81 (1.39–2.35)	1.63 (1.29–2.05)	1.71 (1.43–2.03) ***
CIN 1	88	43	138	84	2.03 (1.41–2.92)	1.63 (1.25–2.14)	1.77 (1.42–2.20) ***††
Non-attenders							
ICC	0	4	8	18	N/A	0.45 (0.20–1.04)	0.37 (0.17–0.83)
CIN 3 or AIS	28	38	42	42	0.75 (0.46–1.23)	1.02 (0.66–1.56)	0.89 (0.65–1.23) ***
CIN 2	54	50	46	39	1.10 (0.75–1.62)	1.20 (0.78–1.84)	1.15 (0.86–1.52) ***
CIN 1	29	25	37	39	1.19 (0.69–2.02)	0.97 (0.62–1.52)	1.05 (0.75–1.48) ***

N/A=not applicable.

Significant association between age and histological outcome is given in Table as P-value *0.01<0.05, **0.001 < 0.01 and *** <0.001.

Significant effect modification between age and screening method is given in Table as P-value †0.01<0.05, ††0.001 < 0.01 and ††† <0.001.

Adapted from Leinonen et al. BMJ 2012;345:e778

Table 13. *Continued*

	No of cases Age 25–34 years		No of cases Age ≥ 35 years		Hazard ratio (95% CI)		
	HPV screening	Conventional screening	HPV screening	Conventional screening	Age 25–34 yrs	Age ≥ 35 yrs	Overall
Colposcopy referral							
ICC	1	1	8	6	0.72 (0.05–11.5)	1.43 (0.50–4.12)	1.21 (0.45–3.24)*
CIN 3 or AIS	37	20	80	54	1.50 (0.87–2.58)	1.97 (1.39–2.78)	1.81 (1.35–2.43) ***
CIN 2	85	59	106	85	1.17 (0.84–1.62)	1.66 (1.25–2.20)	1.52 (1.22–1.89) ***
CIN 1	47	26	70	52	1.46 (0.91–2.36)	1.79 (1.25–2.56)	1.72 (1.29–2.29) ***†
Intensive screening							
ICC	0	0	3	0	N/A	N/A	N/A
CIN 3 or AIS	23	2	32	14	4.61 (1.09–19.6)	2.59 (1.38–4.86)	2.97 (1.70–5.18) ***
CIN 2	64	11	70	15	2.33 (1.23–4.43)	5.29 (3.03–9.25)	4.45 (2.93–6.78) ***
CIN 1	34	6	49	21	2.27 (0.95–5.42)	2.65 (1.59–4.41)	2.66 (1.72–4.10) ***
Negative/normal result							
ICC	0	1	5	1	N/A	4.93 (0.58–42.2)	2.50 (0.49–12.9)
CIN 3 or AIS	2	10	4	9	0.22 (0.05–1.00)	0.44 (0.13–1.42)	0.32 (0.13–0.79) ***
CIN 2	6	15	14	16	0.44 (0.17–1.13)	0.86 (0.42–1.77)	0.65 (0.37–1.13) ***
CIN 1	7	11	19	11	0.70 (0.27–1.80)	1.70 (0.81–3.58)	1.18 (0.67–2.09) ***†

N/A=not applicable.

Significant association between age and histological outcome is given in Table as P-value *0.01<0.05, **0.001 < 0.01 and *** <0.001.

Significant effect modification between age and screening method is given in Table as P-value †0.01<0.05, ††0.001 < 0.01 and ††† <0.001.

Adapted from Leinonen et al. BMJ 2012;345:e778

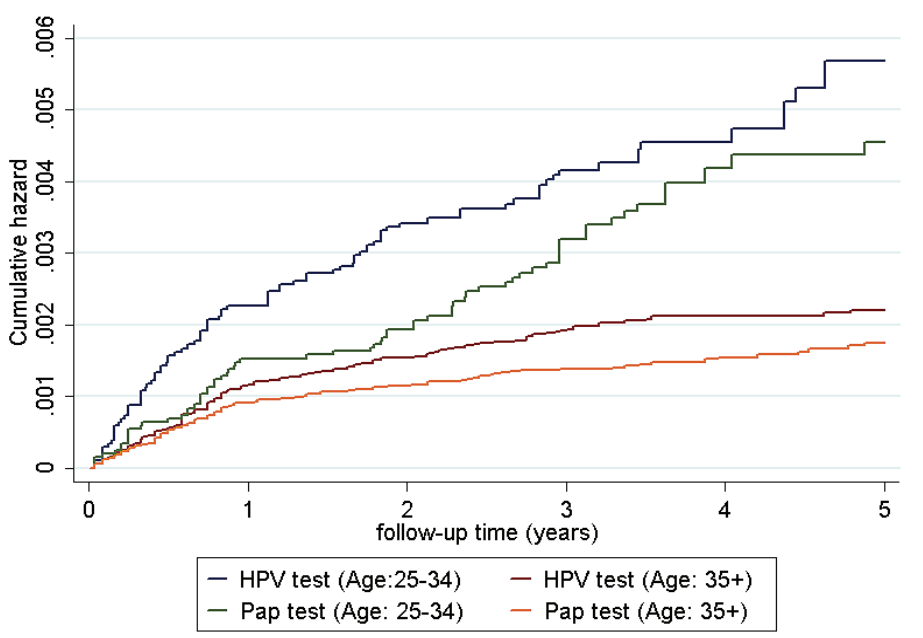
The hazard rate ratio of CIN 3 or AIS was 1.32 (95% CI 1.09-1.59) among all invited women and 1.62 (1.28-2.06) among attenders in the HPV vs. the conventional screening arm. The hazard ratio estimates of CIN 1 and CIN 2 lesions were even slightly higher than those of CIN 3 or AIS after the primary HC2 test in comparison with the primary cytology in respective subgroups. Also, the detection of invasive cervical cancers was non-significantly increased after screening with the primary HC2 test compared to the primary cytology. Among non-attenders, there were fewer invasive cervical cancers diagnosed in the HPV than in the cytology arm during follow-up (HR 0.37, 95% CI 0.17-0.83) whereas no difference between screening arms was observed for less severe cervical lesions (any CIN or AIS, Table 13).

The HR of CIN 3 or AIS was 1.81 (95% CI 1.35-2.43) among women referred to colposcopy and 2.97 (1.70-5.18) among those women who were recommended to participate in intensive screening in the HPV vs. the conventional screening arm. Generally, the detection of all grades of cervical precancerous lesions was increased after the HPV DNA test than after the cytology both among the women referred to colposcopy and among those who were recommended for intensive screening at the index screen. The detection rate of CIN 3 or AIS was significantly lower in HC2-negative women in comparison to women with normal cytology (HR 0.32; 95% CI 0.13-0.79). However, the detection of CIN 2 lesions was not significantly decreased (HR 0.65; 95% CI 0.37-1.13) and no decrease at all was observed for CIN 1 (HR 1.18; 95% CI 0.67-2.09) after a negative result in the HC2 test compared to a normal result in the cytology. There was no significant difference in the detection rates of the ICC between the two screening arms by status at the index screen. The hazard ratio estimates for all histological outcomes and all statuses at the index screen are given in Table 13.

For 25- to 34-year-olds, the cumulative hazard of CIN 3 or AIS per 10 000 person years during the five-year period was 57 (95% CI 45–72) in the HPV screening arm and 46 (35–59) in the conventional arm. For women aged ≥ 35 years, the cumulative hazards were 22 (19–26) and 17 (14–21) per 10 000 person years, respectively (Figure 13).

The cumulative hazard of CIN 2 in women <35 years group was 120 (95% CI 110-140) and 80 (66-96) per 10 000 person years in the HPV vs. the conventional screening arm. The cumulative hazards of CIN 2 at older age groups were substantially lower using both screening methods, 37 (32-42) in the HPV screening and 23 (19-27) per 10 000 person years in the conventional screening arms among women 35 years and older. In principal, the cumulative hazard of CIN 1 was similar to that of CIN 3 or AIS at the respective age group and using the same screening method (data not shown). Among women aged 35 or more there was only a rather small absolute increase in the cumulative hazard of a mild and moderate pre-cancerous lesion in the HPV arm compared with the conventional arm.

CIN 3 or AIS



CIN 2

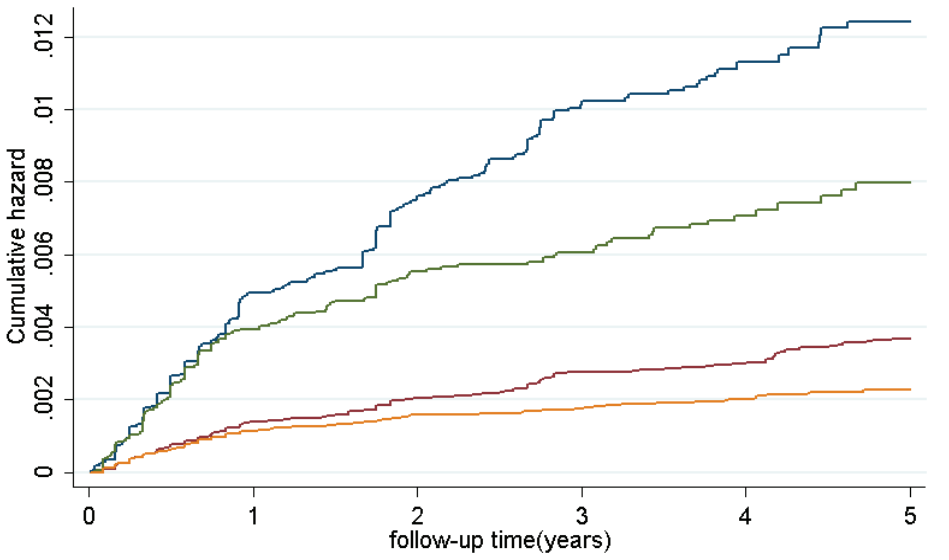


Figure 13 *The cumulative hazard of cervical precancerous lesion among all women invited for cervical screening in the Finnish HPV screening trial in 2003–2007 by age group at randomisation and screening arm.Redrawn from Leinonen et al.BMJ 2012;345:e778.*

10. Discussion

10.1 Risk factors for cervical hrHPV infection (I)

10.1.1 Sociodemographic factors

We found that the age of the woman was strongly associated with the hrHPV infection. Our study therefore supports the existing evidence that the prevalence of HPV infection is age-related (Petignat et al. 2005, IARC 2007, Klug et al. 2007, Bardin et al. 2008, Coupe et al. 2008, Kjaer et al. 2008, Nielsen et al. 2008, Agorastos et al. 2009, Arbyn et al. 2009a, Shipitsyna et al. 2011, Ucakar et al. 2012). Apart from age, the prevalence ratios of covariates differed somewhat between a univariate and the fully adjusted regression model in our study. This finding emphasises the need to adjust for age when other sociodemographic covariates are also modelled.

Marital status most likely reflects sexual behaviour. In our study, unmarried and divorced women showed increased level of risk for an hrHPV infection compared to married couples. This finding is in line with previous reports which have consistently reported that unmarried, divorced or separated women carry up to a three fold risk of contracting cervical HPV infection compared to married couples (de Sanjose et al. 2003, Herrero et al. 2005, Ronco et al. 2005, Kahn et al. 2007, Stamataki et al. 2010).

Although based on only a few highly multiparous women, we did not find any association between the number of births and hrHPV infection. This is consistent with existing literature which argues that the number of full time pregnancies is indeed a risk factor for cervical cancer, but the risk may not be HPV-mediated (Muñoz et al. 2002, Hinkula et al. 2004, Castellsague et al. 2006, IARC 2012).

A study of 2110 women from Colombia found that two areas had particularly high numbers of HPV infections and these were cities in which much of the economy relied on tourism. It was therefore assumed that people living in these cities were more prone to engage in high risk sexual behaviour (Camargo et al. 2011). Our study, however, did not find a significant association between HPV infection and a geographic region. Our trial included three big cities and six smaller ones from the Uusimaa district in Southern Finland. Crude Risk Ratios suggested that big cities had higher hrHPV prevalence rates but following adjustments by other variables, municipality was not related to hrHPV prevalence. Other sociodemographic features were more likely to explain the observed differences seen at the univariate level. It is also possible that municipalities in our study just were too similar to show significant differences. On the other hand, the effect of the place of residence or the type of residence may be challenging to demonstrate as HPV infection has been associated with both rural areas and a low socioeconomic status (SES) (de Sanjose et al. 1996, Parikh et al. 2003, Khan et al. 2005a, Kahn et al. 2007, Stamataki et al. 2010, Drolet et al. 2013) and urban areas where an increased risk for high risk sexual activities is more prevalent (Camargo et al. 2011, Miranda et al. 2012).

10.1.2 Hysterectomy

Our results suggested that hysterectomised women were at an increased risk (RR 1.37; 95% CI 1.09-1.72) for hrHPV infection and, thus, possibly at a risk to develop other HPV-related malignancies (Kalliala et al. 2005, de Martel et al. 2012). Castle et al. studied 573 women who had undergone a hysterectomy and 581 age-matched controls with intact cervices using the PCR method. Contrary to us, they found no differences in prevalence rates of hrHPV infection among hysterectomised women compared to non-hysterectomised women (Castle et al. 2006). They also concluded that hysterectomised women have a minimal risk of HPV-induced cancer and therefore are unlikely to benefit from HPV testing.

A study from Costa Rica using the PCR method compared HPV types detected in cellular specimens from the vagina and cervix of 353 women. In that study, any HPV infection was more frequently detected in vaginal specimens than in cervical specimens. This was mainly due to the increased vaginal prevalence of low-risk HPV types (Castle et al. 2007). The HC2 test cross-reacts with certain non-carcinogenic HPV types which are phylogenetically related to types in a probe mixture (Castle et al. 2008, Sargent et al. 2010) so an increased prevalence of a vaginal HPV infection might explain our results among hysterectomised women.

It is not likely that our result would be explained so that the hysterectomy would have been the treatment option for the CIN. Based on the Finnish Current Care Guidelines hysterectomy is used treating CIN only in exceptional situations with troublesome recurrent high-grade cervical lesions (Finnish Current Care Guidelines 2010). However, as multiple covariates were analysed, it is also possible that the result occurred due to chance. This finding needs to be re-evaluated in the future using our genotyping data.

10.2 HPV prevalence (I and IV)

10.2.1 High-risk HPV infection

The mean age of HC2 tested women was 45.2 years and the prevalence of any hrHPV infection in the Finnish screening population varied from 7.5 to 7.8% (I, IV). The highest proportion of hrHPV infection was 24% using the HC2 test and 16% using the PCR method among women 25- to 29-olds. This was in line with previously reported data (Cuzick et al. 2003, Cuschieri et al. 2004a, Petignat et al. 2005, Bardin et al. 2008, Coupe et al. 2008, Hibbitts et al. 2008, Kjaer et al. 2008, Ronco et al. 2008, Sargent el al. 2008, Arbyn et al. 2009a, Bruni et al. 2010, Ucakar et al. 2012). In a cohort of Finnish university students, 33% of all female students had a prevalent lower tract genital HPV infection using the HC2 test. 84% of all the infections were of hrHPV types. The results are in line with our findings even though the mean age of students was 22.7 years and the study was based on a voluntary attendance (Auvinen et al. 2005).

Among HC2-positive women, a rather constant decline in the proportion of Group 1/2A HPV types was seen between the different age brackets. These varied from the highest value of 66% among women <35 years to 41% among women aged ≥65 years. We found a highest proportion of Group 2B HPV types in the middle-aged (age groups from 40 to 50) bracket and for non-carcinogenic Group 3 types there was a second increase in their proportion from the age of 50 onwards (IV). The bimodal curve is not usually observed in countries with effective cervical screening programmes (Cuzick et al. 2006, Bruni et al. 2010). The results from a large Guanacaste cohort showed that the proportion of cytologic abnormalities was 14% among those HPV-positive women who were aged 54 years or older, whereas it was 27% within the age group of <35 years and 28% among women aged 35 to 54 years. This suggests that a positive HPV test result at older ages does not reflect on cytologic abnormalities in the similar way as it does at younger ages (Kovacic et al. 2006). This finding was also made in our study among HC2-positives (IV).

10.2.2 HPV types

The most common HPV type among women screened by the primary HC2 test during 2003–2005 was HPV 16 (IV). The proportion of HPV 16 infections (0.9%) in our study was the same as reported in the population-based sample from Spain (de Sanjose et al. 2003). However, it was evidently lower than reported in other individual European studies among women attending a cervical cancer screening (Forslund et al. 2002, Cuschieri et al. 2004a, Ronco et al. 2005, Klug et al. 2007, Bardin et al. 2008, Coupé et al. 2008, Hibbits et al. 2008, Kjaer et al. 2008, Sargent et al. 2008, Agorastos et al. 2009, Arbyn et al. 2009a, Shipitsina et al. 2011, Monsonego et al. 2012, Ucakar et al. 2012). It was also somewhat lower than reported in a meta-analysis of women with normal cytology in countries of Northern and Western Europe (de Sanjose et al. 2007). The difference may be due to the different analytical sensitivity of the HPV DNA detection methods supported by the fact that only 57% of the initially HC2-positive samples contained carcinogenic HPV DNA (Group 1/2A) in our study. Also, it may reflect the effect of cervical screening in which cervical lesions containing the HPV 16 are removed from screening population at early phases (Coupe et al. 2008).

The next frequent types after HPV 16 were HPV 31 (0.7%) and HPV 52 (0.5%) (IV). Among women with a normal cytology, HPV 52 is the second most common type in Africa and the third most common in Asia and worldwide (de Sanjose et al. 2007, Bruni et al. 2010). It is also among the third most common types in Eastern Europe and in Denmark. However, in the rest of Europe it is not (de Sanjose et al. 2007, Bardin et al. 2008, Kjaer et al. 2008, Bruni et al. 2010, Shipitsina et al. 2011, Ucakar et al. 2012). The proportion of HPV 18, which is the second most common type in Europe, was generally lower than in the rest of Europe. This was in line with studies from Eastern Europe (Bardin et al. 2008, Shipitsina et al. 2011, Ucakar et al. 2012) suggesting that the HPV type distribution found in Finland is consistent with that of the geographic region.

HPV 16, together with other types of α9 species, accounted for 38% of all HC2-positive samples and 55% of samples with a positive result when genotyped by the PCR method. This is consistent with a Greek study which reported a similar HPV type distribution following a positive HC2 test (Agorastos et al. 2009). However, substantially higher proportions of infections with α9 types among HC2-positives have been reported from Denmark which could be attributed to age (women aged 20–29 years) and to higher hrHPV prevalence in the Danish population overall (Kjaer et al. 2008, Kjaer et al. 2010). Generally, the relative contribution of HPV 16 correlates inversely with the overall HPV prevalence. This means that in areas where HPV is extremely common (i.e. Africa), the relative contribution of HPV 16 among HPV-positive women is lowest (Bruni et al. 2010).

10.2.3 HPV infection and its correlation to findings in cytology triage

In our study, the proportion of Group 1/2A HPV types was about 50% when cytology was classified as normal and almost 80% when cytology was indicative of a need for a referral to a colposcopy. Several other studies have also demonstrated that the proportion of Group 1 types increase with the increasing abnormality of cytology (Cuschieri et al. 2004a, Klug et al. 2007, Hibbits et al. 2008, Kjaer 2008, Sargent et al. 2008, Arbyn et al. 2009a, Shipitsina et al. 2011, Monsonego et al. 2012, Ucakar et al. 2012).

We found that HPV 16 and related types of α9 species were detected most often among women with LSIL+ cytology except for HPV 52 and HPV 58 which were more frequent among women with ASCUS. Two other studies have also reported higher proportions of HPV 52 among women with a borderline cytology than among those with dyskaryotic smears (Cuschieri et al. 2004a, Hibbits et al. 2008). It is possible that this relates to different classification systems of cytology, particularly in our study in which Papanicolaou classes were converted to TBS 2001. Finally, we showed that Group 2B HPV types were merely related to ASCUS cytology and CIN 1 lesions whereas other studies had not assessed them separately.

30% of the initially HC2-positive samples were negative after the PCR amplification (IV). In the previously mentioned Greek study, authors reported that up to 27% of the HC2+ samples could not be genotyped due to an inadequate cellular content (Agorastos et al. 2009). This corresponds to a proportion of PCR-negative/HC2-positive women in our data and may be due to the known drawback of the HC2 test (Castle et al. 2008, Sargent et al. 2010). However, the proportion of PCR-negative/HC2-positive samples was only about 5% in the Danish study (Kjaer et al. 2010). This suggests that the proportion of PCR-/HC2+ -results depends on the age of the women to be screened and on the PCR method used. The finding that there were cervical precancerous lesions detected among women who were HC2-positive but negative in HPV genotyping indicates a different performance and clinical validity of the test methods.

We demonstrated that PCR-/HC2+ -results mainly attribute to women with a normal or borderline cytology which is in agreement with two population-based screening studies. The Dutch study showed that increasing the HC2 cutoff from 1.0 to 3.0 relative light units (RLU/Co ratio) would decrease the HC2-positive results related to normal or mild

cytological abnormalities. The same study found that then the HC2 and the PCR test results resemble each other (Hesselink et al. 2006). Another study from the UK stated that raising the threshold to an RLU/Co ratio of ≥2 would not only reduce HC2-positivity related to normal and borderline/mild cytology, but also decrease colposcopy referrals, particularly among women aged 35 to 64 years (Sargent et al. 2010). These results were reflected also in your study which found that fewer HC2+ samples were confirmed by the PCR among older women compared with women under age of 35 years (IV).

10.2.4 HPV infection and its correlation to findings in histology

Consistent with other studies, the proportion of Group 1/2A HPV types increased with an increasing CIN grade (Kjaer et al. 2008, Klug et al. 2007, Guan et al. 2012, Monsonego et al. 2012). Concerning the type-specific data, the proportion of HPV 16 and HPV 52 types increased whereas that of HPV 51 decreased with increasing CIN grade. Similar results were reported from the Dutch study (Klug et al. 2007). As with our data, the results were based on small numbers because CIN 3+ is a rather rare outcome in screening studies and, thus, may be attributed to chance. On the other hand, the recent worldwide meta-analysis of 115 789 HPV-positive women showed a substantial reduction in the proportion of HPV 51 by cervical disease grade (Guan et al. 2012). Principally, the most prominent HPV types and their proportions in CIN 3+ lesions were similar to those suggested by the international meta-analyses except for the noticeably low rate of HPV 18 and HPV 45 (Smith et al. 2007, de Sanjose et al. 2010, Li et al. 2011, Guan et al. 2012).

HPV 16 and HPV 18 are the most common types found in ICC. HPV 18 has been preferentially found in adenocarcinoma but new data indicates that HPV 16 and 18 have similar contributions in ADC (de Sanjose et al. 2010, Li et al. 2011). In our data, including all cervical lesions detected in 12 months after the index screen, two out of three cancers were ADCs. They were not, however, associated with HPV 18 (IV). A cross-sectional Swedish study triaged 1 595 women with LSIL cytology using the HPV test. Authors conferred an increased risk for CIN 3+ among LSIL women who were HPV 16-positive but not among those who were HPV 18 and HPV 45-positive compared with no HPV triaging at all (Soderlund-Strand et al. 2011). Both results are consistent with the data suggesting that CIN 3+ lesions among HPV 18-positive women appear in a delayed manner (Khan et al. 2005b, Kjaer et al. 2010) and studies using CIN 3 as a main outcome underestimate the relative carcinogenic potential of HPV 18 and 45 (Guan et al. 2012).

Genotyping for HPV 16 identified better those women who were at a risk for the presence of CIN 3+ than cytology triage at the threshold of LSIL+ at the index screen (IV). A large cohort study of 20 514 women attending cervical screening at the Kaiser Permanente showed that this also remained during follow-up. The 10-year cumulative incidence of CIN 3+ among HPV 16-positive women aged 30 years and older was 20% whereas it was 11% among women with LSIL cytology at a baseline (Khan et al. 2005b).

An HPV-associated PAF in cervical cancer describes the proportional reduction in the rate of cervical cancer incidence that would occur if a particular HPV type was eliminated from the population e.g. by vaccination. Previously, a large Finnish serological case-cohort study found that 61% of SCCs but only 6% of CIN 3 lesions were attributable to the HPV 16 infection (Laukkanen et al. 2010). Instead, we estimated the HPV 16-associated PAF to be 55.8% in CIN 3+. Since we only had three invasive cancers, the proportion of CIN 3 lesions attributable to HPV 16 is apparently close to our estimate among the Finnish screening population. It is likely that the serological study has underestimated the PAFs due to the low clinical sensitivity of serology, especially in cervical precancerous lesions.

10.3 HPV screening (II and III)

Our study is the only HPV screening trial that is conducted as a part of a routine screening programme. Also, it was the first one that used the design of a cytology triage for HPV-positives. We demonstrated that the cross-sectional specificity and Positive Predictive Values of the HPV DNA test alone were inferior to cytology. However, the specificity and PPVs of the HPV DNA test with the cytology triages were at the level of conventional cytology. Importantly, the cross-sectional specificity of HPV screening with the cytology triage was even slightly better than that of cytology among women 35 years and older. This cross-sectional data from the index screen was not affected by the opportunistic screening and it was consistent with previous reports. Numerous studies have proved that HPV testing is more sensitive but less specific for detecting cervical precancerous lesions and cancer than conventional cytology (Arbyn et al. 2006, Koliopoulos et al. 2007, Cuzick et al. 2008, Zhao et al. 2010, Whitlock et al. 2011, Arbyn et al. 2012b).

With regards to the higher clinical sensitivity of HPV screening, the results were in line with previous studies as we detected more CIN 1 and CIN 2 lesions in the HPV screening arm than in the conventional arm (II). HPV screening with cytology triage resulted also in an increased detection of CIN 3 lesions in comparison to cytology. The difference between the screening arms, however, was not statistically significant (RR 1.22; 95% CI 0.78-1.92). Several possible explanations for this exist.

First, women were referred to colposcopy based on the cytology triage equal to LSIL+ in our study. In other studies, colposcopy was usually indicative when HPV-positive women had ASCUS+ cytology or in some cases after a positive HPV test result alone. Second, our reporting included results only from the index (first) screen visit whereas e.g. the NTCC and the ARTISTIC studies had also included results from the follow-up tests in their baseline data (Ronco et al. 2006a, Ronco et al. 2006b, Ronco et al. 2008, Kitchener et al. 2009). Third, the implementation of HPV screening was not blinded which may have affected the diagnostic criteria for cytopathologic and histopathologic interpretation which in turn may have resulted in an increased detection rate of mild and moderate lesions. Last, the results were based on a limited number of histologically confirmed cases of CIN 3+ (n=76).

An equal number of women were referred to colposcopy in both screening arms at the index screen visit (II, III). However, an important issue in HPV screening is how to manage HPV-positive women with a normal cytology who have a substantial future risk of CIN 3+ (Sherman et al. 2003, Dillner et al. 2008, Katki et al. 2011). In our study, these women were recommended to take part in intensive screening after 12 to 24 months as were also those women with a borderline cytology and / or normal result in a colposcopy.

Recommendations for intensive screening were made substantially more in the HPV screening arm than in the conventional arm (II, III). The HPV test with cytology triage was a more effective way of identifying women at risk for a cervical lesion in comparison to cytology. This is mainly attributable to the fact that the number of follow-up tests significantly increased the relative detection rates of all grades of cervical precancerous lesions over one screening round compared to the cross-sectional baseline data. Similar results were reported from the POBASCAM and the HPV FOCAL studies (Bulkmans et al. 2004, Ogilvie et al. 2012, Rijkaart et al. 2012a), but to a somewhat smaller extent.

Among the women who were HPV+ but cytology negative, there were 58 cases of CIN 3+ whereas there were only 16 cases after a borderline cytology within the conventional arm (III). Furthermore, the detection rate of CIN 2 lesions among those women who were initially HPV+ but cytology negative was much higher than the detection rate after a negative or borderline cytology within the conventional arm. This may be partly due to the study protocol as all HPV positive women were re-screened after one to two years and those persistently HPV-positive were referred to colposcopy after two or three consecutive positive results even if the cytology was normal. Also the women with a borderline cytology in the conventional arm were re-screened. Two or three consecutive borderline results triggered a referral to colposcopy.

Across all age groups, the relative detection rates of cervical lesions during the 1st screening round in our study were slightly higher than reported in the POBASCAM study but similar to those reported in other RCTs (Naucler et al. 2007b, Naucler et al. 2009, Ronco et al. 2010, Rijkaart et al. 2012a) except for the ARTISTIC trial (see introduction). The HPV FOCAL study reported 244 cases of subsequent CIN 2+ per 1 000 tested women in the HPV arm and no cases in the control arm following the index screen visit whereas other RCTs have not reported separate risk estimates for follow-up tests (Ogilvie et al. 2012).

Our hazard ratios of CIN 2 (HR 4.45, 95% CI 2.93-6.78) and CIN 3 or AIS lesions (2.97; 1.70-5.18) among the women who were recommended to take part in intensive screening in study III were close to the estimates of women aged 25–34 years in the NTCC Phase 2 in which all HPV-positive women were referred to colposcopy at the baseline (Ronco et al. 2008). Similar hazard estimates suggest that many of the re-screening tests in our study likely have resulted in a colposcopy during 1st screening round. The difference in detection rates among women recommended to take part in intensive screening between study arms was remarkable. It is not likely that all the lesions that had been missed during the index screen would have been prevalent ones. There were probably also new HPV infections in the screening population which are less likely to progress over one screening round. To avoid overdiagnosis, a screening algorithm that follows a positive HPV DNA test result is important.

We used three different health care registers to obtain the best possible information on cervical lesions detected following the initial screening visit. Comparably, in the Swedescreen and the POBASCAM trials both cytology and histology results were retrieved from nationwide registries of cytopathology and histopathology including also non-organised smears and biopsies (Naucler et al. 2007b, Rijkaart et al. 2012a). Presumably there is no prominent underregistration of cervical lesions in either of these studies. The slightly higher risk estimates for high-grade cervical lesions over one screening round in our study may be due to the high opportunistic screening activity in Finland. Also, we cannot completely rule out that the opportunistic screening may have been more intensive after a positive HPV test result than after an abnormal cytology.

RCTs have demonstrated that a higher clinical sensitivity of the HPV DNA test protects from invasive cancer and detects high-grade cervical lesions, i.e. CIN 2+ or CIN +3 lesions earlier than cytology (Naucler et al. 2007b, Kitchener et al. 2009, Naucler et al. 2009, Ronco et al. 2010, Rijkaart et al. 2012a, Arbyn et al. 2012b). We assessed the cumulative detection rates of cervical lesions among all invited women over one screening round. This approach estimates the true effect of the screening method in the screening population strongly suggesting that not all invited women participate in the screenings.

We found that very few cases of CIN 3 or AIS were diagnosed later than three and half years after invitation in the HPV screening arm among women ≥35 years old (III). Contrary, there was a rather constant increase in the detection of CIN 3 or AIS in the conventional screening arm between two and five years following the index screening. This difference between the arms indicates an earlier diagnosis of high-grade cervical lesions by HPV screening as reported in other RTCs. Nevertheless, the increased amount of CIN 2 lesions diagnosed during the 1st screening round does not necessarily mean a significant reduction in CIN 2 lesions at later screenings (Naucler et al. 2007b, Ronco et al. 2010, Rijkaart et al. 2012a). Thus, the higher clinical sensitivity of HPV-based screening for preinvasive cervical lesions may result in an excess of non-progressive CIN 2 lesions but more data on the 2nd screening round results is needed to assess the issue.

In comparison to older women, both screening arms had more CIN lesions among 25- to 34-years olds both at the initial screening visit and during the 1st screening round (II, III). This was even more prominent in the HPV screening arm. Mild and moderate cervical lesions have a higher tendency for a spontaneous regression particularly at younger ages (van Oortmarssen and Habbema 1991, Syrjänen et al. 1992, Moscicki et al. 2004). Therefore, the risk of an overdiagnosis and overtreatment is higher with CIN 1 and CIN 2 lesions in comparison to CIN 3 being detected and treated. On the other hand, the risk of ICC in the future seems to be equally high after treatment for CIN, regardless of the histopathologic diagnosis (Kalliala et al. 2005). It also has to be noted that the low cervical cancer incidence rates currently prevalent in Finland may be due to the highly proactive policy in regards to treatment that was in force before the year 2006 when all CIN 1 lesions were (nearly) always treated. As for the future, it is important to assess whether the excess detection rates of mild or moderate cervical lesions after an HPV screening are accompanied with a decrease in the incidence of the ICC.

We estimated that the cumulative hazard of CIN 3 and AIS was about one third after a negative HPV DNA test result from that of a negative cytology. We also demonstrated

that this decrease was about the same for both young (25-to 34-year-olds at screen) and older women (aged 35 and older). However, there were actually a few more cancer cases detected in the HPV arm after a negative HPV test result than in the conventional screening arm after the negative cytology (III). Our results were in line with the findings of previous studies arguing that the cumulative risk for CIN 3+ is very low in 5+ years post screening among women who had negative results on the HPV test (Dillner et al. 2008, Kjaer et al. 2010, Katki et al. 2011, Kitchener et al. 2011, Arbyn et al. 2012b). At least in countries that currently offer cervical cancer screening every three years, a major benefit of an HPV-based primary screening would be identifying low-risk women in whom an extended screening interval would be appropriate. Allowing for a safe screening interval of 5-7 years for HPV-negative women, 92% of the screened women in our study, could potentially reduce screening demands and thus result in cost savings.

Sensitivity measures a screening test's ability to correctly identify an underlying disease. In all RCTs the sensitivity has been estimated through a detection method that includes both pre-invasive and invasive screen-detected lesions. Sensitivity can also be estimated by an incidence method that is based on the failures of screening to prevent an invasive disease (IARC 2005). The sensitivity of the conventional cytology and the sensitivity of the Finnish screening programme is very high (Nieminen et al. 2004, IARC 2005, Lönnberg et al. 2010). Another study from the Finnish material that compared interval cancer rates after a negative screening test result showed no difference on screening failures between an HPV test with cytology triage and a conventional cytology screening. This also indicates that the sensitivity of cytology and the effectiveness of screening by cytology are high in Finland (Malila et al. 2013).

In contrast, there were slightly more cervical cancer cases diagnosed at the index screen and at the following re-screening visits in the HPV screening arm compared to the conventional screening arm (III). Concerning the cancer outcome, our results are still very preliminary. We have to follow randomised women over two screening rounds, i.e. up to 10 years after the introduction of the study to ultimately assess the effectiveness of HPV screening and the possible overdiagnosis related to it.

10.4 Future challenges

CIN 3, AIS and cancer are rare outcomes among well-screened populations. This finding necessitates a careful assessment of tradeoffs between sensitivity and specificity of the screening tests (Patanwala et al. 2013). Despite the use of the most aggressive referral policy to a colposcopy, the NTCC trial prevented fewer cancer cases than the POBASCAM trial. This is because the baseline cancer rate was lower in the cytology arm in Italy than in the Netherlands. When the outcome is rare, there is less potential for new interventions to improve prevention and therefore more aggressive protocols might be warranted to increase the efficacy of screening (Anttila et al. 2006). This in turn may result in overtreatment and morbidity among women of reproductive age (Kyrgiou et al. 2006, Jakobsson et al. 2007, Simoens et al. 2012, Heinonen et al. 2013). With regards to possible overtreatment, it would be important to study new management strategies. For

instance, surveillance could be a more appropriate approach for CIN 2 lesions, specifically at young ages when the progression of precancerous lesions is less common. Also, CIN 2 lesions could be genotyped and treated immediately, but only involving those women with the presence of HPV 16 or HPV 18 DNA.

It is also worth noting that HPV is an infectious agent and preterm delivery is often caused by chorioamnitis due to an ascending bacterial infection. Thus, it is also possible that a persistent HPV infection as well as CIN and preterm delivery all represent the same phenomenon: a failure in controlling the immune response. More research on the genes controlling the immune response and genetic susceptibility is warranted.

In terms of cervical cancer prevention, one relevant question to arise is whether the colposcopically invisible lesions detected by multiple or random biopsies are of clinical importance. In the U.S. the sensitivity for very small CIN 3 lesions, that constitute most of the disease nowadays, are considered important (Schiffman et al. 2011). Generally, CIN 3 lesions associated with invasion are larger than those without invasion and thicker than the lesions found only through a random biopsy. Regular screening enables to detect these small lesions at subsequent screenings and, thus, allows time for spontaneous regression. This in turn minimises the chance of possible overtreatment. The slow development of ICC also explains the success of the conventional cytology screening. This is due to the fact that those cervical lesions that are possibly missed at the screen are still pre-invasive at the next screening round.

To date, many developed countries have started to vaccinate adolescent girls against HPV 16 and HPV 18. Current HPV16/18 vaccines have demonstrated excellent efficacy against all CIN 3+ lesions although these have been based on a rather short term follow-up period of 4-years (Lehtinen et al. 2012). Vaccines not only prevent HPV16/18 related lesions but also provide a cross-protective efficacy against four carcinogenic non-vaccine HPV types namely those of HPV 31, 33, 45 and 51 (Wheeler et al. 2012). However, vaccines do not protect against all ICCs, not all women will be vaccinated and achieving the full effect of vaccine protection will take a few decennia because the vaccination target groups are young girls. Thus, screening will still be needed in the future. An important issue to address now is how to optimally combine the HPV vaccination and the cervical cancer screening.

When the vaccinated birth cohorts will enter into screening programmes, the prevalence of cervical abnormalities will decrease. This in turn will very likely decrease the performance of the subjective Pap test due to the monotony of reading negative smears. An HPV test followed by cytology triage increases the number of smears that have a high probability of containing relevant cytological abnormalities, and therefore seems to be the most attractive screening strategy also in the vaccine era (Huh et al. 2010). It has been shown that the Positive Predictive Values of cytology and HPV testing decrease when HPV16/18 vaccinated cohorts enter into the screening programmes and, thus, they might warrant tailored screening algorithms (Coupe et al. 2008, Lehtinen et al. 2012).

Currently, two thirds of the annual diagnoses of any CINs in Finland are found outside the national screening programme due to an opportunistic screening which is more expensive and less cost-effective (The National Institute for Health and Welfare 2011).

When considering a nationwide introduction of primary HPV screening, it would be essential to simultaneously reduce the opportunistic screening especially for young women among whom mild cervical lesions and spontaneous regression are common (van Oortmarssen and Habbema 1991, Syrjänen et al. 1992, Moscicki et al. 2004). At the same time, one major challenge faced by the programmes is how to improve attendance at the organised screening as cervical cancers are more often diagnosed among non-attenders than attenders (IARC 2005, Lönnberg et al. 2012, Snijders et al. 2013). A study from Finland demonstrated that self-sampling, i.e. providing self-taken vaginal cells taken at home for an hrHPV detection in the laboratory, is a feasible option that enhances the overall number of screenings and yet is still part of the organised programme. If the self-sampling is used as a third intervention after a written invitation and a reminder letter, the overall attendance level would most likely reach the desired 80% (Virtanen et al. 2011). Thus, self-sampling HPV tests seem a promising approach in recruiting original non-attendees into the screening programmes in developed countries and facilitate access to screening for women in low resource areas (Virtanen et al. 2011, Snijders et al. 2013).

10.5. Strengths and limitations

The results represented in this study were obtained from one of the largest HPV screening trials and from the only one that is specifically designed to assess the effectiveness of a national cervical screening programme. Using the infrastructure of the organised programme, HPV screening was compared to screening with a conventional cytology in an individually randomised setting when most biases should be avoided. The attendance rate in the study area was about 65%, which is somewhat lower than the average for the entire country, but typical for Southern Finland. All of the major analyses were conducted by random allocation (intention to screen) which is a valid approach for public health oriented estimates. However, the same limitation involves our study and most of the other studies using screening material which may not be optimal for etiological research. A cohort study of 80 000 women from rural India provided strong evidence that self-selection in a decision to attend screening correlates with risk factors and thus influences the estimates of risk factors (Thulaseedharan et al. 2013). There are also some other limitations in these studies.

The intended lack of blinding is definitely the most potential cause of bias. First, cytotechnicians, cytopathologists, colposcopists, and pathologists all had access to the screening test results. The awareness of the HPV test result may have affected the diagnostic criteria because the interpretation of both cytology and histology is subjective, and this may partially explain the higher referral and detection rates as seen in the HPV screening arm. However, this is obvious to occur whenever the HPV screening is incorporated into routine health care. One of the study aims was to compare the different screening methods and all the potential effects in routine practice for which the blinding was not considered beneficial. Longitudinal data for the women who participated in the trial will ultimately reveal the possible size of the bias.

Second, according to the protocol, the screening method was not mentioned in an invitation letter but it was disclosed and explained at the screening visit. However, cervical cancer incidence rate was substantially lower in the HPV screening arm than in the conventional arm among non-attenders. This suggests that some women may have received information, e.g. by contacting the sample-taking unit, about the screening method before their decision to participate. This information could have then led to a decision to attend. It was impossible to assess the kind of information women had received about the screening tests beforehand, and whether this had influenced their decision to accept the HPV test or decline it and opt for a Pap test instead. As for future, if greater interest towards HPV testing among those women at a higher risk for cervical cancer exists, then this may result in better attendance rates and even in better efficacy of the screening overall if the test gets accepted as standard screening method.

Another explanation would be that something in the process of randomisation would have failed. Serious efforts such as independent checking of the randomisation procedure, accumulation of women in the study arms over the years and the checking of previous screening history of women were made. However, no technical errors in the randomising process were identified. Moreover, a fail in the randomisation should also be reflected in the number of lesions detected before the study period, but no such difference was observed. Also, a pure chance cannot be ruled out even if the difference was formally significant.

Women who were HPV-positive but had a negative result in cytology triage or women who were negative for any test were not referred to colposcopy at the index screen. The reason is that the ultimate aim of the whole on-going trial is to compare programme effectiveness between the HPV and the cytology screening methods by comparing interval cancer rates between the screening arms. Therefore, it is desirable that possible false-negative lesions remain undisclosed. However, the situation results in a verification bias and our study may have overestimated relative sensitivity and underestimated relative specificity when comparing these parameters to studies with multiple testing or studies in which longitudinal data is available. Unbiased test accuracy parameters can be obtained after a follow-up until the subsequent screening round in five years´ time. Taken into account the high clinical sensitivity of the HC2 test, the very low incidence of CIN 3+ after a negative result on the HPV test, and the high quality of cytology (including a low-proportion of false-negative smears) in the Finnish programme (Lönnberg et al. 2010), the verification bias is likely to be small. Also, it is unlikely to have been affecteded by the comparability of the screening arms because it existed in both.

It should also be noted that the purpose of the randomisation is to assure that the two groups are similar in terms of all the other characteristics except for the intervention in question. However, this only applies in the beginning of the study and the non-blinded randomisation does not guarantee that comparison between the two groups remains intact during the study. In one study (III), we assessed cumulative disease rates over one screening round. These cumulative rates included screen-detected cervical lesions and lesions detected based on a mixture of intensive screening (initiated by the programme) and opportunistic testing (conducted outside the screening programme). In such a setting, opportunistic Pap testing would preferentially improve the Pap test performance because

the more sensitive primary screening test (the HPV test) would identify more diseases to be treated and removed from the study arm. This effect leads to a different occurrence of precancerous lesions between the arms and favours disease detection in the screening method with the higher residual incidence during follow-up. The bias is not controlled by randomisation because the difference was created afterwards. Since there still was a higher detection of lesions in the HPV screening arm than in the conventional screening arm, this bias does not explain our result concerning the cumulative rate at five years.

In two studies (III, IV) we used three different health care registries to obtain all the possible cervical precancerous lesions detected among the study population. In the HDR, every contact made with the health care unit is recorded with some ICD-10 diagnosis. For this reason, it is practically impossible to distinguish between incident cervical lesions and the prevalent ones (Lönnberg et al. 2011). Therefore, we chose the most severe diagnosis from the HDR and accompanied it with the earliest date of admission that included any diagnosis of a cervical lesion, irrespective of its grade. Furthermore, we tried to minimise over-reporting by excluding those cervical lesions that were prevalent at the beginning of the study. Some over-reporting is still likely to remain, however. Fortunately, due to the randomisation the level of over-reporting should be similar in both screening arms.

Considering the study on the type-specific HPV proportions (IV), we did not do genotyping directly but the samples were stored in the STM buffer at −20 ◦C for five to seven years instead. From approximately 3% of the samples no human β-globin DNA could be amplified. The long-term storaging (years instead of months) of the samples may have affected the integrity of the nucleid acids stored in the denaturising liquid (Holland et al. 2003, Anchordoquy and Molina 2007). To assure that the frozen samples were usable for the study, we did a pilot study for a small subset of samples two years earlier. The results from the pilot study (data not shown) did not significantly differ from the ones reported here, suggesting that storage has not markedly influenced DNA preservation. There might have been a lack of material for the DNA extraction and HPV typing. The tubes may not have been closed properly before freezing and some DNA is inevitably lost during the extraction process.

It was also a limitation that we did not genotype HC2-negative samples. The proportions from our study were slightly lower than suggested by international meta-analyses of women with a normal cytology even though we did not exclude women with findings in a cytology triage and histology. Taken into account the great analytical sensitivity of the HC2 test, genotyping the HC2-negative samples would have unlikely changed our results or affected the comparability of the type-specific proportions, however.

The role of multiple infections was not possible to assess due to some technical issues in data handling. The multiple infections were dealt with by adjusting the regression model estimates for all the other HPV types and those women who had not been infected with a particular HPV type (i.e. women with other HPV infections or without any HPV type) were used as the referent group. However, the confounding role of multiple infections was evident as the PAF of CIN 3+ for any HPV type (a model adjusted only for age) was lower than that for Group 1/2A HPV types (adjusted for age, Group 2B and Group 3 HPV types).

Finally, many of the known risk factors for an HPV infection and for a CIN or cancer development, e.g. sexual behaviour or smoking, were not possible to evaluate as such data is not collected and registered as part of routine screening practice. Hormonal status is needed for the interpretation of the Pap results. The information on the use of oral contraceptives, a hormonal replacement therapy or an IUD was asked at the screening visit. However, that information does not take into account the previous use in case of cessation or the length of time in case of continuing hormonal use and, therefore, was not considered usable for the purposes of this study.

10.6 Summary and conclusions

The use of the HPV DNA test during primary screening was evaluated within the population-based screening programme for cervical cancer. The study was a randomised trial on public health services so the results are directly applicable for routine use. The study was based mainly on preinvasive lesions and three different health care registers were used to obtain the best possible information on cervical lesions detected in Finland.

Our study showed a similar inverse relationship between the prevalence of hrHPV infection and age as in other developed countries. However, the highest proportion of less carcinogenic and non-carcinogenic HPV types appeared in the middle age bracket or therafter. These infections were not associated with findings in cytology triage.

From the limited number of risk factors that were tested, age was a strong determinant of the prevalence of hrHPV infection. Other significant risk factors for hrHPV infection included marital status and hysterectomy.

Type-specific HPV proportions among the women who attended cervical screening were lower than reported in other individual European studies and also suggested by international meta-analyses. The most common hrHPV type was HPV 16, followed by HPV 31 and HPV 52. The proportion of HPV 18 was lower than indicated in other studies. The distribution of the hrHPV types in Finland was closer to reports from Eastern Europe than from countries of Northern or Western Europe, suggesting that the HPV type distribution found in Finland is consistent with the regional HPV distribution around the world. However, the prevalence rate of any hrHPV infection, that reflects the background risk for cervical cancer, was not markedly lower than in other European countries. This indicates that the low burden of cervical cancer in Finland is due to prevailing health care actions including free public screenings within the organised programme.

Our study showed that the cross-sectional performance of the primary HPV screening with cytology triage was similar or even better to that of the conventional screening for precancerous lesions. Among women aged 35 years or older, HPV screening with cytology triage was not only more sensitive but also more specific for detecting cervical precancerous lesions in the cross-sectional setting than in the conventional practice. The cross-sectional performance indicates that both screening methods can be used in a population-based screening programme.

Primary HPV DNA test with cytology triage detected clearly more cervical precancerous lesions than cytology within one screening round. Among women aged 35

years or older, we observed a very few cases of CIN 3 or AIS diagnosed later than three and a half years after invitation within the HPV arm. By contrast, there was a rather constant increase in the detection of CIN 3 or AIS in the conventional arm between two and five years. This difference in the detection rates indicates an earlier diagnosis of high-grade cervical lesions with the HPV DNA test. As for the future, it is important to assess whether the excess detection rates of mild or moderate cervical lesions after an HPV screening are accompanied with a decrease in the incidence of the ICC.

One of our most important findings was that after a negative result at the index screen visit, the cumulative hazard of CIN 3 or AIS after the HPV screening was about a third of the cumulative hazard after the cytology screening over a screening round. This decrease was roughly the same for all women regardless of age, suggesting that longer screening intervals could be applied for HPV DNA test negatives. This vast majority of women (92% of the screened in our study) have a better protection against future CIN 3 and most likely against invasive cancer too compared to conventionally screened women.

To summarise, the HPV DNA test is more sensitive for precancerous lesions and detects cervical lesions earlier. It also has a higher Negative Predictive Value which allows longer screening intervals after a negative result than cytology. However, HPV-based screening might bring a risk of increased detection of non-progressive lesions. This requires that, when considered for routine use, age groups and screening intervals need to be carefully selected. Particularly, this applies to the screening intervals and algorithms of intensive screening that follows a positive HPV DNA test result. Thus, a gradual implementation of HPV screening in other regions in Finland would be preferred.

Moreover, new HPV tests are introduced in the market all the time but only a subset of them has documented clinical performance for any of the standard HPV testing indications. We analysed the same cervical samples using a signal amplification method (Hybrid Capture 2) and a target amplification method (MGP-PCR). The high proportion of PCR-/HC2+ -results, particularly in women with normal cytology, indicates a different performance and clinical validity of the tests. This emphasises that any new screening test considered for cervical screening should be promptly evaluated within the programme in comparison with the routine test, usually cytology.

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