Aline Simen-Kapeu

Epidemiological Study of Tobacco Use and Human Papillomavirus

Implications for Public Health Prevention

Academic dissertation

To be presented with the permission of the Faculty of Medicine of the University of Tampere, for public examination in the Auditorium of Tampere School of Public Health, Medisiinarinkatu 3, Tampere, on September 11th, at 12.00.

To my beloved family – and my wonderful husband and daughters, Abida Danielle Kapeu and Olive Lanpeerbas Kapeu

"The fear of the Lord is the beginning of wisdom......................…" (Proverbs 9:10).

Abstract

Cervical cancer (CC) is one of the most common forms of cancer in women. The sexually transmitted oncogenic human papillomavirus (HPV) types are the necessary etiological agents of CC. However, only a small fraction of HPV-infected women go on to develop cancer. Other avoidable co-factors that act in conjunction with HPV to promote cervical malignant lesions need to be verified and tackled. Tobacco exposure, a potential environmental cofactor of CC, has attracted increasing attention since the early 1980s.

My aim in this thesis was to assess the role of tobacco exposure in cervical precancerous lesions and cancer of the uterine cervix as well as in multiple HPV infections, and to evaluate the impact of tobacco smoking on the immune response to natural HPV infection as well as to HPV vaccination.

A population-based case-control study of CC was nested within a joint cohort of five Nordic serum banks from Finland, Iceland, Norway and Sweden. The samples of cases and controls were analyzed for cotinine (a biomarker of tobacco smoking) and antibodies to HPV types 16 and 18, herpes simplex virus type 2 (HSV-2), and *Chlamydia trachomatis* (*C. trachomatis*). Due to small sample size, the first study material (171 cases and 496 controls) (Paper I) had limited power to distinguish whether tobacco smoking was an independent cofactor in cervical carcinogenesis or whether its relative role was due to residual confounding by the oncogenic HPVs. In the second study material (Paper I), we assembled almost four times bigger independent material, including 588 cases and 2,861 controls. We identified smoking as an independent risk factor for CC. A highly significant 2- to 3-fold increased risk of invasive CC (Odds ratio (OR) = 2.1; 95% confidence interval (CI): 1.4–3.2) and squamous cell carcinoma (SCC) (OR = 2.7; 95% CI: 1.7–4.3), free of residual confounding bias, was found among HPV16/18-seropositive heavy smokers. In addition, the point estimates increased with increasing age at diagnosis (probably indicating longer exposure) and increasing cotinine level.

In a cross-sectional analysis (Paper II), we compared the association between tobacco smoking and chewing and the risk of multiple HPV infections and cervical squamous intraepithelial lesions (SILs) in two populations with different routes of tobacco exposure. We studied 2,162 women from Côte d'Ivoire, West Africa, and 419 women from Finland, Northern Europe, with baseline data on cervical screening, smoking and chewing habits, HPV DNA status, C. trachomatis status and human immunodeficiency virus (HIV) seropositivity. In both settings, tobacco

consumers (chewers or smokers) ≥30 years of age tended to have an increased risk of low-grade SIL (LSIL). Among tobacco chewers (Côte d'Ivoire), the risk of high grade SIL (HSIL) was five times higher in both young (OR = 5.5, 95% CI: 1.2–26) and older (OR = 5.5, 95% CI: 2.1–14) women compared to non-chewers. We found an increased, albeit not significant, risk of both LSIL and HSIL, in HPV-DNA positive women ≥30 years of age and actively exposed to tobacco through smoking or chewing. There was no increased risk of multiple HPV infections among tobacco consumers.

In a cohort study (Paper III), we evaluated the association between humoral immune response to HPV and smoking in 191 HPV infected women prospectively followed-up for 10 years by cytology and HPV DNA analyses. The baseline sample and the last follow-up sample were analysed for serum cotinine levels, Immunoglobulin (Ig) A and IgG antibodies to HPV16 and 18, and *C. trachomatis* using ELISA methods. Young women (<30 years of age) who smoked were less likely to either seroconvert or maintain detectable HPV16/18 antibodies over the follow-up time (up to 10 years) than non-smokers. This suggests that among young women with oncogenic HPV16/18 infections, smoking impairs the humoral immune response to high-risk HPV types.

A phase III double-blind, randomized controlled trial enrolled 4,808 16- to 17-year-old females in Finland to receive either the prophylactic HPV16/18 AS04-adjuvanted vaccine-like-particle (VLP) vaccine or hepatitis A vaccine (Havrix™) as a control (Paper IV). We conducted a pilot study among 216 participants to compare HPV16/18 antibody levels of nonsmokers and smokers 7 months post-vaccination (one month post the third vaccination dose). Baseline and month 7 serum samples were analysed for cotinine levels and IgG antibodies to HPV16 and 18. We found that women who smoked appeared to have comparable levels of anti-HPV16 and 18 antibodies to nonsmokers at month 7 post-vaccination. Our data suggest that smoking may not have an impact on the humoral antibody response following HPV vaccination.

Alongside the development and combination of HPV vaccination programmes and screening and early diagnosis of CC, our findings support public health initiatives intended to prevent tobacco smoking and chewing exposures, particularly among young women.

Keywords: human papillomavirus, seroepidemiology, tobacco smoking, tobacco chewing, immune response, fertile-aged women, HPV vaccine, cervical cancer prevention

Abstract in Finnish

Kohdunkaulan syöpä on yksi naisten yleisimmistä syöpämuodoista. Seksin yhteydessä tarttuvat syöpävaaralliset ihmisen papilloomavirukset (HPV) ovat kohdunkaulansyövän välttämättömiä syytekijöitä. Vain pieni osa HPV-infektioon sairastuneista naisista saa syövän. Muut vältettävissä olevat osatekijät, jotka saavat yhdessä HPV:n kanssa kohdunkaulansyövän esiasteet etenemään on identifioitava ja voitettava. Tupakointi, mahdollinen ympäristöperäinen kohdunkaulansyövän osatekijä, on herättänyt kasvavaa mielenkiintoa 1980-luvulta lähtien. Tarkoitukseni oli tässä työssä määrittää tupakoinnin rooli kohdunkaulansyövässä ja sen esiasteissa sekä HPV-infektioissa, ja arvioida tupakoinnin vaikutusta luonnollisen HPV-infektion ja toisaalta rokotuksen seurauksena syntyneelle immuunivasteelle.

Väestöpohjainen upotettu tapaus-verrokkitutkimus hyödynsi viittä pohjoismaista (Islanti, Norja, Ruotsi, Suomi) seerumipankkikohorttia. Tapausten ja verrokkien näytteistä tutkittiin kotiniini (tupakoinnin biomarkkeri) ja HPV, herpes simplex virus ja klamydia vasta-aineet. Pienen otoskoon vuoksi ensimmäisen tutkimusmateriaalin mahdollisuudet erottaa onko tupakointi kohdunkaulan syövän kehittymisen itsenäinen riskitekijä vai selittyykö osuus syöpävaarallisten HPV:n jäännössekoitusvaikutuksella (osajulkaisu I) ei ollut mahdollinen. Toiseen tutkimusmateriaaliin (osajulkaisu I) keräsimme neljä kertaa suuremman, erillisen, materiaalin, jossa oli 588 tapausta ja 2861 verrokkia. Osoitimme tupakoinnin olevan kohdunkaulansyövän itsenäinen riskitekijä. Löysimme erittäin merkitsevän 2–3-kertaisen kohdunkaulansyöpäriskin (vaarasuhde, OR = 2.1; 95 % luottamusväli (CI): 1.4–3.2) ja levyepiteelisyöpäriskin (OR = 2.7; 95% CI: 1.7–4.3) HPV16/18 vasta-ainepositiivisten aktiivisten tupakoitsijoiden joukossa, joka oli vapaa jäännössekoitus-vaikutuksesta. Lisäksi piste-estimaatit olivat sitä suurempia mitä korkeampi tutkittavien ikä oli diagnoosihetkellä (so. mitä pitempi altistuminen) ja mitä korkeampi heidän kotiniinitasonsa.

Tekemässämme poikkileikkaustutkimuksessa (osajulkaisu II) vertasimme tupakoitsijoiden ja purutupakan käyttäjien useiden HPV infektioiden ja kohdunkaulansyövän esiasteiden (SIL) riskiä kahdessa populaatiossa. Tutkimuksessa oli 2 162 naista Norsunluurannikolta Länsi-Afrikasta ja 419 naista Suomesta, Pohjoi-Euroopasta, joista oli käytettävissä tiedot kohdunkaulansyövän seulontatutkimuksesta, tupakoinnista ja purutupakan käytöstä, kohdunkaulan HPV DNA löydöksistä, klamydia ja HIV vasta-ainetiedot. Molemmissa tutkittavien ryhmissä tupakka-altistus (purutupakan käyttäjillä ja tupakoitsijoilla) liittyi kohonneeseen matala-asteisen

SIL-muutoksen riskiin sekä nuoremmilla (OR = 5.5, 95% CI: 1.2–26) että vanhemmilla (OR = 5.5, 95% CI: 2.1–14) tutkittavilla. Löysimme, ei-tilastollisesti merkitsevästi, kohonneen matala- ja korkea-asteisten SIL-muutosten riskin yli 30-vuotiailla tupakka-altistuneilla (purutupakan käyttäjät tai tupakoitsijat) tutkittavilla. Heidän useiden HPV-infektioiden riskinsä ei ollut kohonnut.

Kohorttitutkimuksessa (osajulkaisu III) arvioimme tupakoinnin liittymistä kiertäviin HPV vasta-aineisiin 191 HPV-infektioon sairastuneella naisella, joita seurattiin aina 10 vuotta kohdunkaulansyövän seulontanäytteillä joista määritettiin myös HPV-DNA.Tutkimuksen alku- ja loppuhetkillä otetut näytteet tutkittiin kotiniini, ja HPV16 ja HPV18 immunoglobuliini (Ig) G ja IgA vasta-aineiden suhteen, myös klamydia vasta-aineet määritettiin ELISA-menetelmällä. Nuoret tupakoitsevat naiset serokonvertoivat HPV16/18 viruksille tai säilyttivät nämä vasta-aineensa 10 seurantavuoden ajan vähemmän todennäköisesti kuin tupakoimattomat. Tämä viittaa siihen, että nuorilla naisilla, joilla on syöpävaarallisten HPV16/18 virusten aiheuttama infektio, tupakointi huonontaa vasta-aineresponssia korkean riskin HPV-virustyypeille.

Kaksoissokkoutettuun, satunnaistettuun faasi III tutkimukseen osallistui Suomessa 4808 16–17-vuotiasta naista, ja he saivat joko HPV16/18 AS04-adjuvantoitua viruksen kaltaisista partikkeleista (VLP) koostuvaa rokotetta tai kontrollina hepatiitti A rokotetta (HavrixTM) (osajulkaisu IV). Teimme pilottitutkimuksen 216 tutkittavan joukossa vertaamalla HPV16/18 vasta-aineita tupakoimattomilla ja tupakoitsijoilla 7 kuukautta rokotuksen aloittamisesta (kuukausi kolmannen rokotuskerran jälkeen). Alkuhetken ja 7 kuukauden seeruminäytteistä analysoitiin kotiniini ja HPV16 ja HPV18 IgG vasta-aineet. Naisilla, jotka tupakoivat ja jotka eivät tupakoineet HPV16 ja HPV18 vasta-aineet olivat samalla tasolla 7 kuukautta rokotuksen jälkeen. Tuloksemme viittaa siihen, että tupakoinnilla ei ole vaikutusta HPV rokotuksen seurauksena syntyvään vasta-aineresponssiin.

Tulostemme mukaan HPV-rokotus ja seulontaohjelmia, ja kohdunkaulansyövän varhaista diagnostiikkaa kehitettäessä myös kansanterveystyö tupakoinnin ja purutupakan käytön vähentämiseksi olisi perusteltua, erityisesti nuorilla naisilla.

Avainsanat: ihmisen papilloomavirus, seroepidemiologia, tupakointi, purutupakan käyttö, immuuniresponssi, hedelmällisessä iässä olevat naiset, HPV rokote, kohdunkaulansyövän ehkäisy

Table of Contents

12 Epidemiological Study of Tobacco Use and   Research 20
      Human Papillomavirus   THL 2009
      Aline Simen-Kapeu

List of abbreviations

AIDS	Acquired immunodeficiency syndrome
APC	Antigen presenting cells
CC	Cervical cancer
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CO	Carbonmonoxide
COHb	Carboxyhemoglobin
CRPV	Cotton-tail rabbit papillomavirus
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
ETS	Environmental tobacco smoke
FMC	Finnish Maternity Cohort
GYTS	Global Youth Tobacco Survey
hrHPV	high-risk human papilloma virus
HC	Hybrid capture
HIV	human immunodeficiency virus
HPV	human papilloma virus
HSIL	high-grade squamous intraepithelial lesion
HSV-2	herpes simplex virus type 2
IARC	International Agency for Research on Cancer
IFN	Interferon
Ig	Immunoglobulin
IMC	Icelandic Maternity Cohort
ISH	In situ hybridisation
LBC	Liquid-based cytology
LSIL	low-grade squamous intraepithelial lesion
MHC	major histocompatibility complex
MONICA	Monitoring trends and determinants in cardiovascular disease
NSMC	Northern Sweden Maternity Cohort
OR	Odds ratio
ORF	Open reading frame
PAP-smear	Papaniculaou-stained cytological cervico-vaginal smear
PATRICIA	Papilloma trial against cancer in young adults
PCR	Polymerase chain reaction
PIN	Personal identification number
RCT	Randomized control trial
RNA	Ribonucleic acid
RR	Relative risk
SCC	Squamous-cell carcinoma
SHS	Second-hand smoke

SIR	Standardized incidence ratio
STP	Smokeless tobacco products
UPR	Upstream regulatory region
VIA	Visual inspection with acetetic acid
VILI	Visual inspection with Lugol´s iodine
VIP	Västerbotten Intervention Program
VLP	Virus-like particle
WHO	World Health Organization

List of original publications

This dissertation is based on the following original articles referred to in the text by their Roman numerals:

I **Simen-Kapeu A**, Luostarinen T, Jellum E, Dillner J, Hakama M, Koskela P, Lenner P, Löve A, Mahlamaki E, Thoresen S, Tryggvadóttir L, Wadell G, Youngman L, Lehtinen M. Is smoking an independent risk factor for invasive cervical cancer? A nested case-control study within Nordic Biobanks. Am J Epidemiol 2009;169:480–488.

II **Simen-Kapeu A**, La Ruche G, Kataja V, Yliskoski M, Bergeron C, Horo A, Syrjänen K, Saarikoski S, Lehtinen M, Dabis F, Sasco AJ. Tobacco smoking and chewing as risk factors for multiple human papillomavirus infections and cervical squamous intraepithelial lesions in two countries (Côte d'Ivoire and Finland) with different tobacco exposure. Cancer Causes Control 2009;20:163–170.

III **Simen-Kapeu A**, Kataja V, Yliskoski M, Syrjänen K, Dillner J, Koskel P, Paavonen J, Lehtinen M. Smoking impairs human papillomavirus (HPV) type 16 and 18 capsids antibody response following natural HPV infection. Scan J Infect Dis 2008;40:745–751.

IV **Simen-Kapeu A,** Surcel H-M, Apter D, Paavonen J, Lehtinen M. Impact of smoking on humoral Immunoglobulin G antibody response to a human papillomavirus type 16 and 18 AS04-adjuvanted virus-like particle vaccine – A pilot study. Manuscript submitted.

These articles are reproduced with the kind permission of their copyright holders.

1 INTRODUCTION

Cervical cancer (CC) is the second most common cancer among women worldwide. The majority (83%) of cases occur in the developing world, where in most countries, CC is the leading cause of cancer mortality among females (World Health Organization (WHO) 2003, Ferlay et al. 2004). Cervical infection with oncogenic human papillomaviruses (HPVs) is the main cause of CC (Walboomers et al. 1999). Of the numerous oncogenic high-risk (hr) HPV types, 70% of CC is attributed to HPV16 and HPV18 (Bosch et al. 2002, Lehtinen et al. 2001). However, infection with HPV cannot be a sufficient cause of CC because of the high numbers of HPV-infected women who do not develop cancer (Walboomers et al. 1999). It is likely that environmental and host-related cofactors act in conjunction with HPV to promote malignant progression of squamous intraepithelial lesions (SIL), which is the clinical manifestation of hrHPV infection.

Tobacco exposure, an environmental factor in human cancers, is of interest in cervical carcinogenesis because of: 1) the correlation of increased incidence lung cancer and CC (Korhonen et al. 1999), 2) the consistent association of smoking with cervical intraepithelial neoplasia (CIN) grade 3 and CC (Plummer et al. 2003), 3) the comparable strength of the association between tobacco smoking and chewing and CC (Rajkumar et al. 2003) 4) biologic plausibility, including the observation of nicotine-derived carcinogens in cervical mucus after smoking (Holly et al. 1993) and 5) the potential for intervention through anti-smoking campaigns (Nandakumar et al. 2005).

For development, evaluation and effective implementation of prevention strategies and therapeutic agents against hrHPV infection and CC, a better understanding of the impact of tobacco exposure on hrHPV infection, associated natural and vaccine induced immune responses, and cervical carcinogenesis is required.

2   Review of the litterature

2.1   Tobacco use

2.1.1 History and epidemiology

The history of smoking starts among the Native Americans who used it for ceremonial purposes 5000 years BC. Christopher Columbus first brought tobacco to Europe from the West Indies in 1492. From the beginning it was used for medical purposes and in history it is mentioned when the Queen of France, Catherine of Medici, was cured from stomach pains by tobacco. She got the tobacco from Jean Nicot and named it "Nicotiana". Soldiers during the great European wars spread the use of tobacco, mostly used as snuff or smoked in pipes. It was not until the Crimean War, in the middle of 19th century, that cigarettes became more common.

When the first cigarette machine was constructed in 1870, cigarette smoking flourished. This was also the start for the big tobacco companies (Goodman 1995). Cigarette smoking was from the beginning a masculine habit and spread among soldiers during World War I and II. Women began to take up smoking during and after World War II, thus putting children in closer contact to environment tobacco smoke (ETS). The era of the well-educated and career-oriented women began; smoking became a sign of independence and was seen as part of women's liberation. Cigarette smoking then spread to other groups in society. While the well-educated women are now dropping the habits because of increased health awareness, it has now become most prevalent among underprivileged women with low education attainment (Torell 2002).

Worldwide, about 50 % of males and 10 % of females are smoking, but there are great variations among nations. In Finland, 26 % of 15 to 64-year-old males and 18 % of females smoked daily in 2005 (Helakorpi et al., 2005). Smoking prevalence among 13 to15-year-olds ranges from 1 % to 40 % in different countries (Global Youth Tobacco Survey (GYTS) Collaboration Group 2002). During the last decade smoking prevalence has decreased in the Western world but increased in the developing countries (Shafey et al. 2003). In the developing countries too, men started smoking first and women followed soon thereafter. Globally, tobacco consumption as well as production is growing and women in Asia and Africa are now the main target group for the tobacco companies (Mackay and Eriksen 2002).

Though smoking prevalence in the Western world is decreasing, smoking has kept an aura of tough and smart glamour, and around 100, 000 new young smokers are recruited daily (Mackay and Eriksen 2002). In total, about 1/3 of the adult population smokes and WHO has calculated that 1000 cigarettes are manufactured per year per person, including women and children (Mackay and Eriksen 2002). A

TABLE 2.1. Prevalence of tobacco use and number of smokers by WHO region and levels of development in 2000

	Prevalence (% of the population ≥15 years of age)			No. of tobacco users (thousand)		
	Men	Women	Total	Men	Women	Total
Who region						
African Region	29.4	7.4	18.4	51,967	13,420	65,387
Region of the Americas	32.0	20.9	26.3	94,035	64,072	158,107
Eastern Mediterranean Region	35.3	6.1	21.0	52,543	8,670	61,213
European Region	44.9	18.7	31.2	150,628	68,545	219,173
South East Asia Region	48.1	5.3	27.3	251,699	26,484	278,183
Western Pacific Region	61.2	5.7	33.8	390,632	35,784	426,146
Level of development						
Developed	33.9	21.2	27.4	114,783	75,891	190,673
Developing	49.8	7.2	28.9	809,725	114,718	924,443
Transitional	54.1	13.9	32.7	82,837	24,153	106,989
World	47.5	10.3	28.9	11,005,927	217,755	1,223,682

From Guindon and Boisclair 2003.

nearly two-fold difference in smoking rates is seen in men across different WHO regions, with the lowest level in the Eastern Mediterranean Region (34.2%) and the highest in the Western Pacific Region (62.3%). Based on these weighted prevalence estimates, there are over 1.2 billion smokers across the six WHO regions, women being in the minority in the developing countries (Table 2.1).

The prevalence of smokeless tobacco use is high among women. In Mumbai, 59% of women used smokeless tobacco (Gupta 1996). The prevalence was similar to that of other South-Asian female populations. Around 49% of UK-Bangladeshi females, 35.5% of Nigeran females and 59% of rural Malesian females use smokeless tobacco (Thomas et al. 2004, Gan 1995, Croucher et al. 2002). Women appear to have a higher prevalence of the chewing habit in many countries of the South due to the belief that tobacco has many magical and medicinal properties; keeping the mouth clean, getting rid of bad smell, curing toothache, controlling morning sickness, and minimizing labour pains (Muwonge et al. 2008).

Tobacco use prevalence can be decreased by a variety of tobacco prevention and control efforts. Reporting on the adverse health effects from smoking the anti-smoking debate was accelerated in the 1980's when it was shown that passive smoking was also a health hazard. During the 1990's numerous conventions, national as well as international, addressed the smoking issue. Educational, clinical, regulatory, economic, and comprehensive approaches are widely used and studied. WHO and European Union, have made up rules and recommendations for how the

"pandemic of smoking" can be defeated. Tobacco control is highly cost-effective (World Bank 1999). Many countries have passed laws on smoke free areas, rules for cigarette commerce and public health interventions to control tobacco use.

As an example in Finland, the Tobacco Control Act was passed as early as in 1976 (Puska et al. 1997). It prohibited smoking in most public places, restricted tobacco advertising, and set a 16-year age limit for tobacco purchases. Further amendements to the Act were made in 1995, when, for example, the age limit for tobacco purchases was raised to 18 years, and in 2000, when ETS was included in the national list of carcinogenic substances. Among Finnish adult males, smoking prevalence is nowadays one of the lowest in Europe (Shafey et al. 2003). In general, the smoking trends suggest that the impact of tobacco policy is decreasing smoking initiation in youth (Helakorpi et al. 2004); for example the legislation appears to have decreased purchases from commercial sources to minors (Rimpelä and Rainio 2004).

2.1.2 Global burden of tobacco use for public health

Tobacco is packed with harmful and addictive substances. Scientific evidence has shown conclusively that all forms of tobacco cause health problems throughout life, frequently resulting in death or disability. Smokers have markedly increased risks of multiple cancers, particularly lung cancer, and are at far greater risk of heart disease, stroke, emphysema and many other chronic diseases. The use of smokeless tobacco causes cancer in humans (International Agency for Research on Cancer (IARC) 2004). Smokeless tobacco contains carcinogens, which contribute to cancers of the oral cavity and the risk of other cancers. Smokeless tobacco use also causes a number of noncancerous oral conditions and can lead to nicotine addiction similar to that caused by cigarette smoking.

Women who smoke suffer additional health risks. Smoking in pregnancy is dangerous to the mothers as well as to the foetus, especially in countries where health facilities are inadequate. Smoking is not only harmful during pregnancy, but has long-term effects on the offspring. This is often compounded by exposure to passive smoking by the mother, father or other adult members (Fenercioglu et al. 2009).

Of today's global population, 650 million will eventually be killed by tobacco. Approximately half of smokers are killed by their habit – a quarter while still in middle age (35-69 years) (Peto et al. 1996). On average, smokers die ten years younger than non-smokers. Tobacco kills more than Acquired Immuno Deficiency Syndrome (AIDS), legal drugs, illegal drugs, road accident, murder and suicide combined (Doll et al. 2004). Tobacco already kills more men in developing countries than industrialized countries, and it is likely that death among women will soon be the same. Annual deaths due to tobacco in industrialized countries were 1.3 million in 1995 and reached 2.1 million in 2001 and will increase further to 3 million by

2025–30, whereas in developing countries this was 0.2 million in 1975, 2.1 million in 2001, and 7 million by 2025–30, which is a very rapid increase (WHO 2008). While 0.1 billion people died from tobacco use in the 20th century, ten times as many will die in the 21st century. The main diseases by which smoking kills people are substantially different in America (where vascular diseases and lung cancer predominate) (Peto et al. 2004), than in China (where obstructive pulmonary disease predominates, causing even more tobacco deaths than lung cancer) (Liu et al. 1998, Niu et al. 1998), or in India (where half of the world tuberculosis deaths take place, and the ability of smoking to increase the risk of dying from tuberculosis is of particular importance) (Gajalashmi et al. 2003). Around the world, it is estimated that there are currently 30 million individuals who start to smoke every year. With current smoking patterns, worldwide mortality from tobacco is likely to rise from 2–3 million deaths a year in 2001 to about 10 million a year around 2030 (WHO 2008). A survey conducted in 1990 in 44 industrialized countries showed that smoking caused an average of 24% of all male deaths – but 35% of these deaths occur in middle age (35–69 years). This proportion was about 12% in Chinese middle-aged men but is expected to rise to about 33% by 2030. Currently, smoking causes 7% of all female deaths overall.

2.1.3 Smoking forms of tobacco use

There is a variety of smoking tobacco products on the world market (Table 2.2).

Cigarette is any roll of tobacco wrapped in paper or other non-tobacco material; filter-tipped or untipped; approximately 8 mm in diameter, 70–120 mm in length.

A *cigar* is any roll of tobacco wrapped in leaf tobacco or in any other substance containing tobacco. There are four main types of cigars: little cigars, small cigars "cigarillos", regular cigars and premium cigars. Some little cigars are filter tipped and are shaped like cigarettes. Little cigars contain air-cured and fermented tobacco and are wrapped either in reconstituted tobacco or in a cigarette paper that contains tobacco and/or tobacco extract. Cigarillos are small, narrow cigars with no cigarette paper or acetate filter. Regular cigars are up to 17 mm in diameter, 110-150 mm in length. Premium cigars (hand-made from natural, long filter tobacco) vary in size, ranging from 12 to 23 mm in diameter and 127 to 214 mm in length (Stratton et al. 2001). The chemical composition of the tobacco leaf is determined by plants genetics, cultivation practices, weather conditions and curing methods (Tso 1991). Cigarettes and cigars use blended tobaccos and the type of tobacco used in these products has a decisive influence on the physicochemical nature of the smoke they produce.

Bidis are the most popular form of smoking of tobacco in India. They are also becoming increasingly popular among teenagers in the USA (Malson and Pickworth 2002). A bidi is made by rolling a rectangular piece of a dried temburni

leaf around approximately 0.2–0.3 g of sun-dried, oriental tobacco and securing the roll with a thread. These cigarettes are perceived by some as a better-testing, cheaper, safer or more natural alternative to conventional cigarettes (Malson et al. 2001, Stanfill et al. 2003).

Chuttas are coarsely prepared cheroots with 2–9 cm long, prepared by rolling local tobacco inside a sun-dried tobacco leaf. They are usually the products of cottage or small-scale industries. Nearly 9% of the tobacco produced in India is used for making *chuttas*. It is estimated that about 3000 million *chuttas* are made annually in India. The term "reverse smoking" is used to describe smoking while keeping the glowing end of tobacco product inside the mouth. Reverse *chutta* smoking is practised extensively by women in the rural areas of Visakhapatnam and the Srikakulam district of Andhra Pradesh (Van der Eb et al. 1993).

A *cheroot* is a roll made from tobacco leaves. *Cheroots* were commonly smoked by both Indian men and women in South India. *Dhumti* is a kind of conical cigar made by rolling tobacco leaf in the leaf of another plant. Unlike *bidis* and *chuttas, dhumtis* are not available from vendors but are prepared by the smokers themselves (Bhonsle et al. 1976).

Kreteks are types of small cigarettes that contain tobacco (approximately 60%), ground clove buds (40%) and cocoa, which gives a characteristic flavour and "honey" taste to the smoke (Stratton et al. 2001). *Kreteks* are indigenous to Indonesia, but are also available in the USA.

Pipe smoking is one of the oldest from of tobacco use. The different kinds of pipes used for smoking range from the small – stemmed European types made of wood to long-stemmed pipes made from metal or other material.

A *hookah* is an Indian white pipe in which the tobacco smoke passes through water before inhalation. It used to be more common among women, the reason being that it was inconvenient for men to carry a *hookah*, whereas women remain at home for most of the time.

Hooklis are clay pipes commonly used in western India. Once the pipe is lit, it is smoked intermittently. On average, 15 g of tobacco is smoked daily. Hookli smoking was common among men in the Bhavnagar district of Gujarat (Mehta et al. 1969).

A *chillum* is a straight conical pipe made of clay, 10–14 centimetres long, held vertically. It is exclusive and common among men and is confined to the northern states of India, predominantly rural areas (Wahi 1968).

2.1.4 Smokeless forms of tobacco use

Smokeless tobacco products have existed for thousands of years among populations in South America and Southeast Asia. Over time, these products have gained popularity throughout the world. Smokeless tobacco is consumed without burning

the product, and can be used orally or nasally. There are different types of smokeless tobacco products (STP) in use around the world. Oral smokeless tobacco products are placed in the mouth, cheek or lip and sucked (dipped) or chewed. Tobacco pastes or powders are used in a similar manner and placed on the gums or teeth. Fine tobacco powder mixtures are usually inhaled and absorbed in the nasal passages.

Smokeless tobacco comes in two main forms: ***chewing tobacco*** (loose leaf, in pouches of tobacco leaves, "plug" or "twist" form) and ***snuff*** (finely ground or cut tobacco leaves that can be dry or moist, loose or portions packed in sachets, and administered to the mouth, or the dry products to the nose or mouth). When administered orally, the tobacco can also be mixed with other psychoactive ingredients.

A list of the wide range of oral and nasal tobacco products used is presented below (Table 2.2).

TABLE 2.2 List of smokeless tobacco products by continent

Common name	Constituents	How used
EUROPE		
Moist snuff, Snus	Tobacco; water; sodium carbonate; sodium chloride; moisturizer; flavouring; nicotine	A pinch (called a dip) is usually placed in the upper gingivolabial sulcus. The average user keeps snus in their oral cavity for 11 to 14 hours per day.
Dry snuff	Tobacco	Inhaled up the nostrill
Nicotine gum (non-pharmaceutical)	Tobacco	Gum to be chewed
Gutkha	Tobacco	Chewed or smoked in pipe
Chewing tobacco	Tobacco	Chewed or smoked in pipe
NORTH AMERICA		
Dry snuff	Tobacco + aromatic oils, spices	Inhaled up the nostril
Loose leaf chew	Leaf tobacco; sweetener and/or liquorice	A piece of leaf is tucked between the gum and jaw, typically toward the back of the mouth. It is either chewed or held in place. Saliva spit or swallowed.
Moist plug	Enriched tobacco leaves; fine tobacco; sweetener and/or liquorice	Chewed or held between the cheek and lower lip. Saliva may be spit or swallowed.
Moist snuff	Tobacco	A pinch "dip" or held between the cheek/gum. Saliva may be swallowed.
Plug chew	Enriched tobacco leaves; fine tobacco; sweetener and/or liquorice	Chewed or held between the cheek and lower lip.1 Saliva may be spit or swallowed.
Twist roll (chew)	Tobacco; tobacco leaf Extract	Chewed or held between the cheek and lower lip. Saliva may be spit or swallowed.
Iq'mik	Tobacco, punk ash	Users pinch off a small piece and chew the iq'mik. Often, the user may premasticate the iq'mik and place it in a small box for later use by others, including children and sometimes teething babies.
SOUTH AMERICA		
Chimo	Tobacco resin; alkaline ash; Paullinia yoco; banana peel; sugar; avocado seed	A very small amount of the paste is placed under the tongue and absorbed there. Saliva is traditionally spat out. Chimo is popular as a replacement for cigarettes and provides a similar bolus of nicotine.
Dry snuff, Rapé	Dry tobacco powder with peppery smell	Sniffed through nostrils

Common name	Constituents	How used
INDIA SUBCONTINENT		
Gul	Tobacco powder, molasses, other ingredients	Often used for clearing teeth
Gutkha	Betel nut, catechu, tobacco, lime, saffron, flavouring, saccharine, mint	Held in the mouth and chewed. Saliva is generally spit out, but sometimes swallowed.
Khaini	tobacco; slaked lime paste; areca nut	Paste is placed in the mouth and chewed
Mawa	Tobacco; slaked lime; areca nut	Placed in the mouth and chewed fo 10 to 20 minutes
Tuibur, hidakphu	Tobacco water	Sipped and held in mouth 5–10 min and then spat out
Mishri (masheri)	Tobacco	Applied to the teeth and gums, often for the purpose of cleaning the teeth. Users then tend to hold it in their mouths (due to the nicotine addiction).
Nass (naswar, niswar)	Nass: tobacco, ash; cotton or sesame oil; water; sometimes gum. Naswar or niswar: tobacco, slaked lime; indigo; cardamom; oil; menthol; water	Held in the mouth for 10 to 15 minutes. Naswar is sometimes chewed slowly
Pan masala	Tobacco; areca nuts, slaked lime, betel leaf.	A quid is placed in the mouth (usually between the gum and cheek) and gently sucked and chewed. Pan masala is sometimes served in restaurants after the meal.
Zarda	Processed tobacco	Along with betel quid
Creamy snuff	Tobacco, clove oil, glycerine, menthol, spearmint, camphor	Often used to clean teeth. The manufacturer recommends letting the paste linger in mouth
Red tooth powder	Tobacco	
MIDDLE EAST		
Shammah	Tobacco; ash; slaked lime	
AFRICA		
Toombak	Tobacco; sodium bicarbonate	Product is rolled into a ball of about 10g called a saffa. The saffa is held between the gum and the lip or cheeks, or on the floor of the mouth. It is sucked slowly for 10 to 15 minutes. Male users periodically spit, while female users typically swallow the saliva generated.
Snuff	Tobacco	Sniffed through nostrils, portion bags introduced

From the European Scientific Committee on Emerging and Newly Identified Health Risks, 2007.

2.1.5 Assessment of tobacco exposure

The selection of a strategy to measure smoking exposure is affected by factors such as: (a) required accuracy of the smoking estimate, (b) need for immediate feedback to the smoker, (c) accurate disclosure by the target population, (d) concurrent use of nicotine replacement therapy, (e) need to distinguish between reduction and abstinence, (f) need for validating smoking status, and (g) availability of resources. The various approaches differ in accuracy, validity, objectivity, ease of measurement approach, acceptability and cost. Research evidence on the measurement strategy should guide the decision to use questionnaire data, a biomarker or both.

2.1.5.1 Self-reports

Self-administered questionnaires are a cheap method of assessing smoking status; they are easy to use with great feasibility (fit within setting) and accomplished by written or verbal communication. In this method, the participant responds to questions, either verbally or in writing, regarding smoking abstinence or cigarette consumption (number of cigarettes smoked, frequency, and duration). Questionnaires are noninvasive for the test subjects and the confidentiality of information reduces the refusal rate among participants. Self-reported information can be used to measure behavioural change, to evaluate the exposure risk or to study pathways to smoking cessation (Bauman et al. 1989, Wills and Cleary 1997). The validity of questionnaire data has been studied (Vartiainen et al. 2002). In special groups (adolescents, pregnant women), self-reported smoking is more likely to be under-reported (Owen et al. 2001, Britton et al. 2004, Burstyn et al. 2009).

2.1.5.2 Biochemical methods

The term biomarker means a measurement that reflects an interaction between a biological system and a chemical, physical, or biological environmental agent (Haufroid and Lison 1998). Biological quantification of tobacco use is based on some aspect of the composition of inhaled tobacco smoke. Tobacco smoke is composed of gaseous and particle components. The gaseous component is made up of room air, carbon monoxide, nicotine and volatilized hydrocarbons such as hydrogen cyanide. The primary particle component of tobacco smoke is tar, which carries nicotine. Substances such as nicotine, cotinine, thiocyanate, carbon monoxide and some minor alkaloids of nicotine have been identified and tested as biomarkers of both active cigarette smoking and second hand smoke exposure (SHS) (Woodward et al. 1991).

Nicotine

The major and most pharmacologically active alkaloid of tobacco is nicotine (Jacob et al. 1999). The amount of nicotine uptake is dependent on a smoker's inhalation behaviours (e.g. deep or long inhalation of smoke) and metabolism of nicotine (Benowitz 1999). Most nicotine is metabolized into cotinine and eventually excreted (see cotinine below). Nicotine may be extracted and measured from blood, saliva, and urine (Benowitz 1996). More recently, it has been measured from samples of hair and toenails (Al-Delaimy et al. 2002). Nicotine as a biomarker agent, however, is of limited use (Velicer et al. 1992). Any assay using nicotine must be very sensitive because of the small amount of nicotine present in body fluids. Furthermore, because of its short half-life (2 hrs) and individual variation in its rate of metabolism (Benowitz et al. 1996, Idle 1990), nicotine levels can be only approximated, and may give a biased estimate of tobacco use/exposure.

Thiocyanate

Tobacco smoke contains high concentrations of hydrogen cyanide gas, which is primarily metabolized into thiocyanate (SCN). Like cotinine, SCN can be measured in blood, urine and saliva (Velicer et al. 1992). The following issues affect the usefulness of SCN as a biomarker. Despite its long half-life (10–14 days), the sensitivity and specificity of the assay method are low. SCN levels are influenced by industrial exposure and dietary intake (almonds, bamboo shoots, sugar cane, cauliflower, broccoli, beer and ale (Benowitz 1999, Woodward et al. 1991). Because of these limitations, determination of SCN has not gained wider use (Scherer and Richter 1997, Velicer et al. 1992).

Carbon monoxide (CO)

Cigarette smoke contains a high concentration of CO in gaseous form. Regular cigarette smoking may produce carboxyhemoglobin (COHb) levels ranging from 5% (1 pack per day) to 9% (2–3 packs per day), whereas heavy cigar smoking can produce COHb levels up to 20%. CO has a half-life of 4–5 hrs in adults and can be measured in both exhaled alveolar air and blood (Stewart 1975). Although CO can be measured by analysis of hemoglobin for COHb using a carbon monoxideoximeter instrument, this approach is not favoured because the procedure to collect the specimen (blood) is invasive. Instead, a much simpler and direct measurement of CO can be accomplished using exhaled air and a simple handheld breath analyzer. This method does not require the samples, such as those of blood, saliva, or urine, to be collected and stored, and only minimal training is needed in using the device.

The immediately available measurement of CO level, which is shared with the smoker, can depict the detrimental effects of smoking. This may affect the smoker's subsequent smoking behavior (Secker-Walker et al. 1997). Thus, CO measurement

has been used as part of anti-smoking campaigns. Researchers have demonstrated high correlations among CO, self-reported smoking and urinary cotinine (Secker-Walker et al. 1997). Exhaled CO has been successfully used to corroborate self-report data, with concordance approaching 100% (Becona and Vazquez 1998).

Environmental sources of CO can result in CO levels indistinguishable from those produced by direct cigarette use, thereby confounding the measurement (Velicer et al. 1992, Becona and Vazquez 1998). Another disadvantage of CO measurement is the relatively short half-life of CO (4–5 hrs). In general population, false-negative rates of CO measurements have been found to range from 2% to 16% (Velicer et al. 1992). In addition, the sensitivity decreases with infrequent and irregular smoking patterns, causing those who are light or atypical smokers to appear indistinguishable from non-smokers (Jarvis et al. 1987, Lando et al. 1991).

Cotinine

Cotinine is a useful and popular biomarker of tobacco use. Most nicotine entering the body (70%–80%) is metabolized into cotinine. Cotinine is present in the blood serum, saliva, urine, amniotic fluid, cervical mucus and hair of both smokers and non-smokers exposed to tobacco smoke. It has been cited as the most useful marker for distinguishing tobacco users from non-users, for estimating the nicotine intake of tobacco users, and for specifying the exposure of nonsmokers to second hand smoke (Benowitz and Jacob 1994). Cotinine has an extended biological half-life (15–40 hrs). Its level in the body is directly related to the quantity of nicotine absorbed during the last few days (Wagenknecht et al. 1990). The presence of cotinine indicates exposure to nicotine, either from environmental exposure or direct consumption.

An advantage of cotinine as a biomarker is its high sensitivity. It can distinguish very low levels, such as from SHS in non-smokers, from levels associated with cigarette smoking. Small amounts of cotinine in the body can result from ingestion of foods rich in nicotine (such as cauliflower, eggplant, potatoes, tomatoes and black tea), but these levels are considered insignificant (Benowitz 1996). Measurement techniques have been developed. Cotinine can be quantified in blood, serum, saliva and urine. Various techniques are used for quantitative analysis including: (a) radio immunoassay, (b) high-performance liquid chromatography, (c) gas–liquid chromatography and (d) gas chromatography combined with mass spectrometry (SNRT Committee for Biochemical validation 2003, James et al. 1998). Woodward and colleagues (1991) compared cotinine levels with those from exhaled CO, self-reported tobacco exposure and thiocyanate. The results showed a high correlation among all the markers for the smoking group, but a lower correlation among the nonsmokers exposed to second hand smoke. The investigators concluded that cotinine is the most accurate discriminator between smokers and non-smokers (Woodward et al. 1991). In other studies, serum cotinine was demonstrated to be

a better measure of cigarette smoking than was questionnaire (Perez-Murray et al. 1993, Stable et al. 1995, Britton et al. 2004, Burstyn et al. 2009).

Exhaled carbon monoxide and cotinine (detected in blood, urine or saliva) are sufficiently sensitive, specific and feasible for general use, and are therefore frequently used as biomarkers of cigarette smoking.

New biomarkers and strategies to detect tobacco exposure

A number of new biomarkers are under development. One new strategy is to use hair specimens for analysis of cotinine in order to assess individuals' smoking history. Although there are limitations, such as confounders caused by hair color, hair dyes and other chemical treatments, a statistically significant correlation between hair cotinine and nicotine intake has been found (Eliopoulos et al. 1996). This strategy is promising because of the noninvasive nature of the test, the lack of stringent specimen storage requirements and the ability to detect a history of tobacco usage in patients who temporarily abstain from smoking prior to testing (Eliopoulos et al. 1996).

Biochemical validation is often considered to be a "gold standard" (= considered more accurate than self-reported habit) in validation studies. When used in combination with self-report, biomarkers provide information about the concurrent validity of self-report, and have been noted to increase the accuracy of self-reporting (Kathleen and Muñoz 2004, Wagenknecht et al. 1992, Clark et al. 1996, Becona and Vazquez 1998).

2.2   Cancer of the uterine cervix

2.2.1 Epidemiology

Cancer of the uterine cervix is the seventh most common cancer overall and the second most frequent cancer in women worldwide (Ferlay et al. 2004; Denny 2008). It is a major cause of morbidity, mortality and premature death among middle-aged women in developing countries, who account for 80% of the annual estimated 493,000 new cases and 274,000 deaths worldwide. In these low resourced countries, cervical cancer accounts for 15% of female new cancer cases, with an absolute cumulative risk of 1.5% before the age of 65 years. In developed countries, these proportions are smaller with only 3.6% of new cancers and a cumulative risk of 0.8%. If effective preventive interventions are not implemented, over 1 million women will suffer from cervical cancer annually by the year 2030 (Denny 2008), leading to a far greater in risk and disease burden in developing countries compared to developed countries, and increasing the social inequalities.

The highest incidence rates are observed in the developing countries in sub-Saharan Africa, Melanesia, Latin America and the Carribean, South-Central and Southeast Asia, with age-standardized (world population) incidence rates ranging from 18.7 to 42.7 per 100,000 (Maucort-Boulch et al. 2008). In more developed regions, these rates are generally lower than 14.5 per 100,000 (Parkin et al. 2005; Parkin 2008). These lower incidence rates have, however, materialized after the introduction of screening programmes in the developed countries in the 1960s. Earlier, the incidence in the developed countries was similar to that of developing countries today in most of Europe, North America and Japan (Gustafsson et al. 1997). Cervical cancer incidences were estimated to be 38.0 per 100,000 in the Second National Cancer Survey of the United States (Dorn et al. 1959), 37.8 per 100,000 in Hamburg, Germany in 1960–62, 28.3 per 100,000 in Denmark in 1953–57 and 22.1 per 100,000 in Miyagi, Japan in 1959–60 (Doll et al. 1966) The lowest rate of cervical cancer, 0.4 per 100,000, has been reported in Ardabil, northwest Iran (Sadjadi et al. 2003). Very low rates have also been observed in Israel (2.0 per 100,000), China (6.8 per 100,000), Western Asia (5.8 per 100,000) and Finland (4.0 per 100,000) (Parkin et al. 2005).

Cervix uteri
Age-Standardized incidence rate per 100,000

< 9.4   < 16.8   < 25.8   < 33.4   < 87.3
GLOBOCAN 2002, IARC
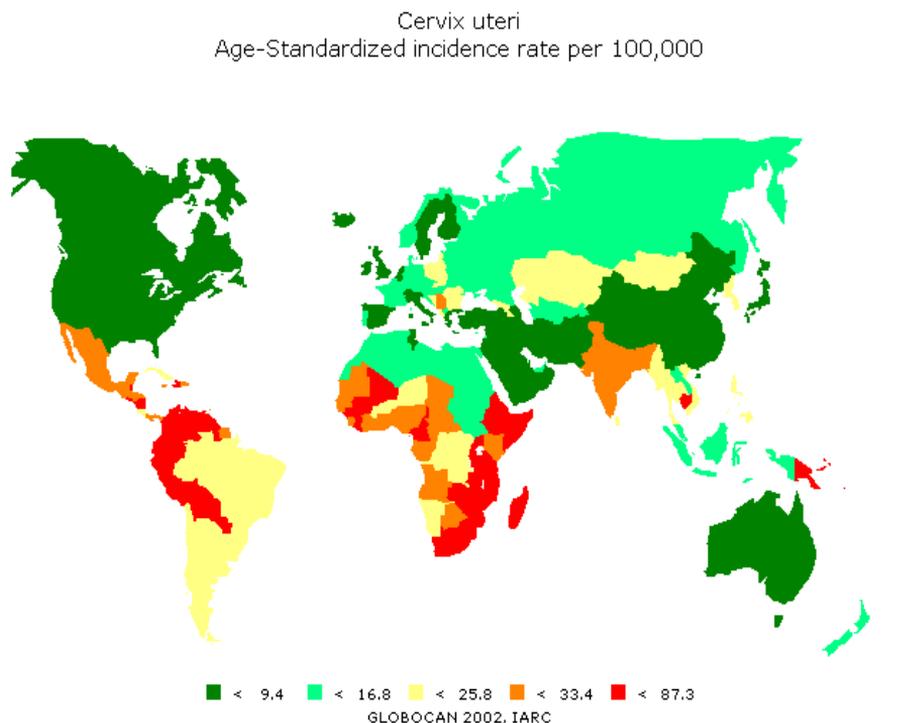
Mortality from cervical cancer is considerably lower than the incidence in both developing and developed countries but the mortality to incidence ratio is higher for the former (57%) than the latter (47%) (Ferlay et al. 2004). Survival rates vary between regions with good prognosis in regions with low incidence (survival obtained from case fatality ratio was 70% for USA, 66% for Western Europe and 65% for Japan in 2002) and fairly good prognosis even in some developing regions (55% in South America and 58% in Thailand) where many cases present at a relatively advanced stage (Parkin et al., 2005). However, poor prognosis is seen in sub-Saharan Africa (21%) (Parkin et al. 2008).

2.2.2 Natural history

Invasive CC is usually preceded by a long phase of pre-invasive, occult disease. This pre-invasive disease is microscopically assessed and characterized as a spectrum of progressive lesion with severity ranging from cellular atypia to various grades of dysplasia or cervical intraepithelial neoplasia (CIN) before progression to invasive carcinoma. Using different terminology systems (Table 2.3), cervical cancer precursor lesions are commonly classified into mild dysplasia or CIN I, moderate dysplasia or CIN II, and severe dysplasia or CIN III. However, the newer terminology of the precursor lesions classifies them as squamous intraepithelial lesions (SILs), which are graded as low (combines condylomatous (HPV) changes and CIN I) and high (encompasses more advanced CIN such as CIN II and III) (Sellors et al. 2003).

Infection of the cervical epithelium with specific hrHPV types plays a fundamental role in the development of cervical cancer through its precursor lesions (Zur Hausen 1999, Whiteside 2008). HPV DNA has been detected in virtually all cervical cancer specimens (Walboomers et al. 1999, Subramanya and Grivas 2008) with HPV16 having the dominating role followed to a lesser degree by HPV types 18, 31, 33 and 45 (IARC 2006). Most cervical abnormalities caused by HPV

TABLE 2.3 Terminology of cervical precancerous abnormalities

Dysplasia terminology	Cervical Intraepithelial Neoplasia (CIN) system	Bethesda system
Unspecified cellular changes	Cellular atypia	Atypical Squamous Cells of undetermined significance (ASCUS)
Mild dysplasia	CIN I	Low-grade squamous intraepithelial lesions (LSIL)
Moderate dysplasia Severe dysplasia/ Carcinoma in situ (CIS)	CIN II CIN III (includes CIS)	High-grade squamous intraepithelial lesions (HSIL)

infections do not progress to high-grade SILs (HSIL) or cervical cancer, but regress spontaneously. The long timeframe between initial infection and overt diseases indicates that other exogenous and endogenous cofactors, such as reproductive factors, other sexually transmitted diseases, smoking, nutritional deficiencies and genetic susceptibility, acting in conjunction with the hrHPVs probably participate in disease progression (Sellors et al. 2003, Stewart et al. 2003, Woodman et al. 2007, Castro 2008). Spontaneous regression of CIN suggests that a lot of women may not be exposed to these cofactors.
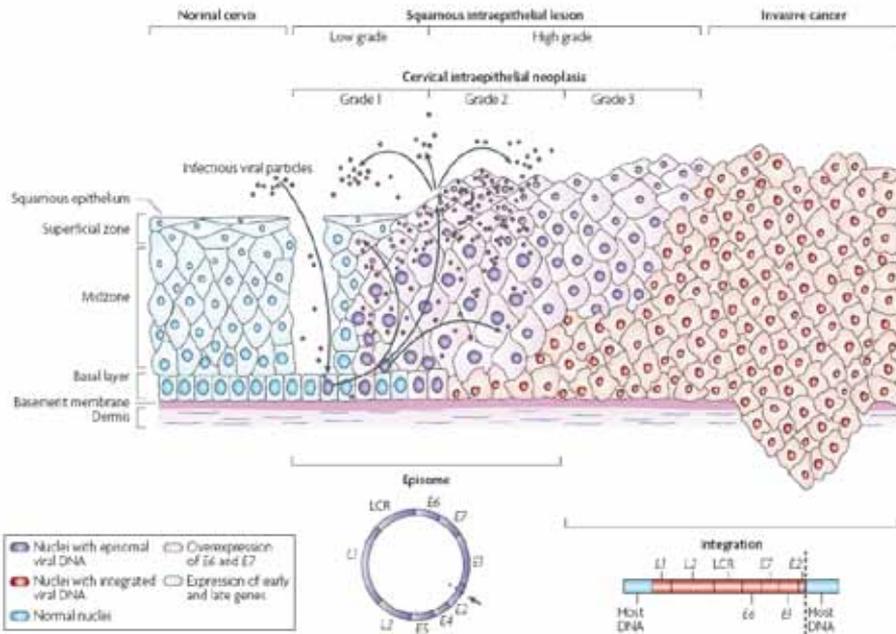
Figure 2.1 Natural history of HPV from Woodman et al. 2007 (Nature Reviews, Cancer)

Studies addressing the natural history of CIN, with particular emphasis on disease regression, persistence and progression, have demonstrated that most LSIL regress to normal within relatively short time-periods or do not progress to severe lesions or invasive disease (Mitchell et al. 1994, Melnikov et al. 1998, Holowaty et al. 1999, Schlecht et al. 2003, Bosch et al. 2008, Wheeler 2008). On the other hand, HSIL has a greater likelihood of progressing to invasive cancer, though a proportion of such lesions also regress or persist (Stanley 2007, Bosch et al. 2008, Wheeler 2008). The mean interval for progression from CIN to ICC ranges between 10 and 20 years.

2.2.3 Risk factors

2.2.3.1 Human Papillomavirus

Structure and classification

Human papillomaviruses belong to the *Papillomaviridae* family. They are small, non-developed, double-stranted DNA viruses with icosahedral symmetry. The virion has a diameter of 55–60 nm and the viral genome is approximately 7900 base pairs long (Chen et al. 1982). The protein coat is composed of 72 capsomers consisting of two structural proteins: one major protein (L1) representing 80% of the total capsid. L2 is the minor protein.

To date, at least 100 HPV types have been classified (de Villiers et al. 2004, IARC 2006). They are classified as genotypes and each type has a given number. The genotypes are based on the sequence homology of the L1 open reading frame (ORF) because this region is well conserved among all members of the papillomavirus family. If the DNA sequence of a new HPV type differs by more than 10% from the closest known papillomavirus type it will be recognized as a new type. A subtype is defined when there is 2–10% difference in the sequence homology. Less than 2% defines a variant (de Villiers et al. 2004).

HPVs are grouped according to the type of epithelia they infect. The majority of HPVs infect cutaneous epithelia or skin. Approximately 40 types infect mucosal epithelia and are called genital HPVs. These types are divided into high-risk types, including cell transformation and low-risk types, causing benign warts, and further divided by sequence homology into A9 (HPV16, 31, 33, 35, 52, 58, 67), A7 (HPV18, 39, 45, 59, 68, 70) (Centers for Disease Control 2008). It has been suggested that at least 14 types are high-risk types (Bosch et al. 1995, IARC 1996, IARC 2006). Main difference between the two major categories is represented by the exclusive capacity of the high-risk HPVs to integrate the host cell's chromosome (Ferenczy and Franco 2001).

Genomic organisation

The genome of HPV contains approximately eight ORFs, which are transcribed from a single DNA strand (Fehrmann and Laimins, 2003). In the upstream regulatory region (URR) which is a non-coding region, sequences for viral replication are found. The gene products can be divided into two classes: early (E) and late (L) proteins (Howley 2006) (Figure 2.1). The early genes are primarily responsible for viral DNA replication, transcription and transformation and the late genes express viral structural proteins that are responsible for maturation and assembly of the virus particle.
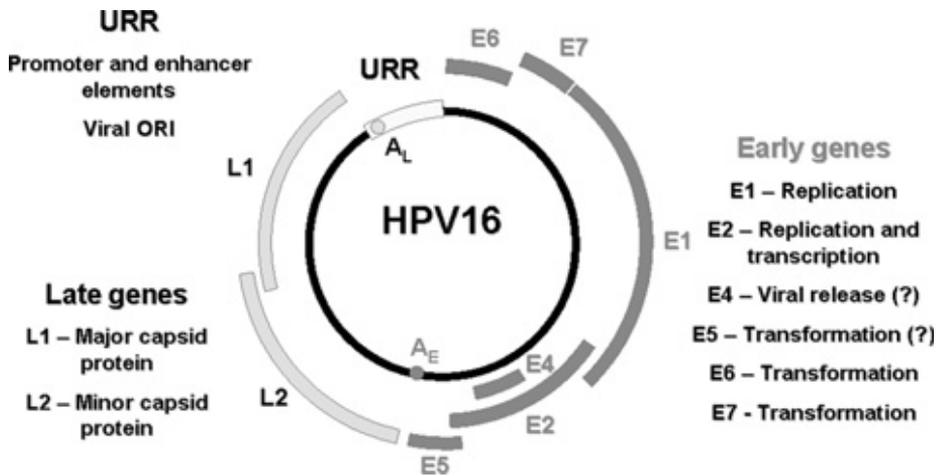
FIGURE 2.2 HPV 16 genome organisation

E1 is the largest ORF in the papillomavirus genome. It is the only papillomavirus protein with defined enzymatic activity (helicase and ATPase activity), which helps viral DNA replication to occur in an efficient manner (Wilson et al. 2002). E1 forms heterodimers with E2, which leads to the initiation of viral replication at the viral origin (Sverdrup and Khan 1995, Horner and DiMaio 2007).

The E2 protein has an important role in the life cycle of papillomavirus because it regulates viral transcription and replication (Lambert 1991). E2 has been shown to induce S-phase arrest, which allows sustained synthesis of viral DNA replication, something that is essential for completion of the viral life cycle (Hamid et al. 2009).

The ORF of E4 is found within the ORF of E2 but has a shorter reading frame. The protein is detected in productively infected cells. The E4 protein is translated from a spliced E1^E4 transcript to form a spliced E1^E4 fusion protein. The pattern of E4 distribution suggests that the E4 function may be required at all stages of the productive cycle (Knight et al. 2004, Doorbar 2006).

E5 is weakly oncogenic in tissue culture assays and improves the effectiveness of the transforming activity of E7 (Bouvard et al. 1994, Valle and Banks 1995). The HPV E5 protein is small, hydrophobic and located mainly at the endosomal membranes, Golgi apparatus and plasma membranes (Burkhardt et al. 1989, Conrad et al. 1993). The protein is probably expressed primarily during the late phase of the life cycle to modulate differentiation-induced functions like viral amplification and late gene expression (Fehrmann et al. 2003, Auvinen 2005).

The E6 and E7 proteins are encoded by all papillomaviruses and their ORFs are located in the 5' part of the early region. Together with E7 from high-risk HPVs, E6 can induce cellular immortalisation of keratinocytes (Hawley-Nelson et al. 1989, Munger et al. 1989). These genes are the main transforming proteins of the high-risk HPV types and act by modulating the activities of the cellular proteins that regulate the cell cycle. The E6 protein is one of the first genes expressed during

HPV infection. It is about 150 amino acids in size and contains two zinc-binding domains with the motif Cys-X-X-Cys. The zinc fingers are important for protein conformation and interaction with DNA. High-risk E6 proteins are found both in the nucleus and in the cytoplasm and have been reported to bind to more than 12 different proteins (zur Hausen 2002, Whiteside 2008). The E7 protein is rather shorter than E6, around 100 amino acids. E7 binds directly to the Rb gene and interferes with the ability of Rb to inhibit cell cycle arrest. This allows productive replication of HPV genes (Fehrmann and Laimins 2003).

L1 and L2 proteins that make up the capsid of the virus are synthesised in the late phase of the viral cycle. The role of the capsid is to protect the genome and to target cellular surface receptors involved in infection. The L1 protein can with or without the L2 protein self-assemble into virus like particles (VLP) when expressed in eukaryotic cells (Kirnbauer et al. 1992, Stanley et al. 2006). The VLPs are morphologically and immunologically comparable to HPV virions.

There is one part of the HPV genome that does not encode any known protein but still has an important function: the long control region (LCR). Its role is to regulate gene expression and replication.

Viral life-cycle

The viral replication cycle is one in which viral infection is targeted at basal keratinocytes but high level expression of viral proteins and viral assembly occur only in differentiating keratinocytes in the stratum spinosum and granulosum of squamous epithelium (Doobar 2007, Frazer 2009) . Although our knowledge is limited in some key areas of the immunubiology and pathogenesis of the viruses, particularly the immediate early events of viral replication, the sequence of events shown in Figure 2.2 (see below), in which viral genes are differentially expressed both temporally and spatially throughout the infectious cycle, is well accepted (Stanley 2006, Wang 2007).
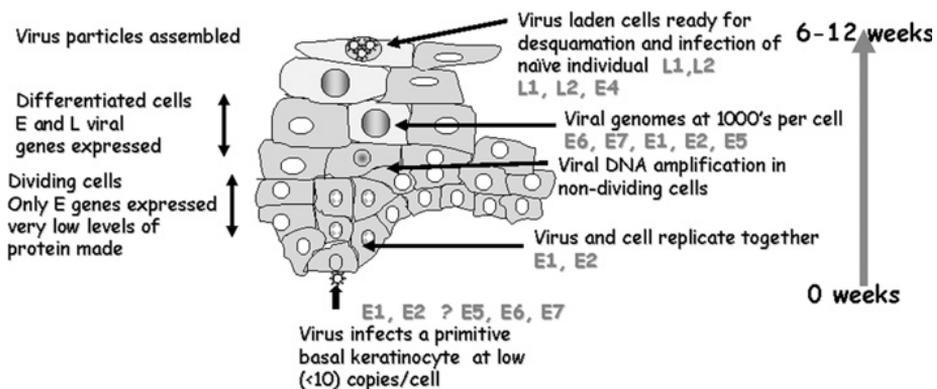
FIGURE 2.3 The HPV infectious cycle (From Stanley 2006)

The replication cycle takes a long time. Even in the best scenario, the time from infection to release of virus will take about 3 weeks since this is the time taken for the basal keratinocyte to undergo complete differentiation and desquamate. In humans the period between infection and the appearance of lesions is highly variable and can vary from weeks to months (Gillison and Shah 2003, Doobar 2007) indicating that the virus can effectively evade the immune system (Stanley 2006, Wang 2007).

The replication cycle is exclusively intra-epithelial, there is no detectable viraemia, viral gene expression is probably confined to keratinocytes and there is no cytolysis or cytopathic death as a consequence of viral replication, assembly and release (Stanley 2006). These key events for the virus occur in the differentiating keratinocyte, a cell destined for death and desquamation far from the sites of immune activity. HPV infection in consequence, is not accompanied by inflammation, there is no obvious 'danger signal' to alert the immune system—a viral strategy that results in persistent, chronic infections as the host remains ignorant of the pathogen for long periods.

HPV immunology

The immune system is complex and its responses can vary from person to person due to different gene disposition and environmental influences. The majority of HPV infected women clear the virus within a rather short period of time. The immune response probably has an important role in clearance of the virus. Understanding immunity to HPV is important in the development of both prophylactic and therapeutic measures against HPV infection and associated cancer.

Antigen presenting cells

Langershans cells (derived from dendritic cells) are the professional antigen presenting cells in the epithelium (Campaner et al. 2006). They present the endocytosed HPV antigens to T-helper cells by using the MHC class II molecules, and thus start up the immune response by secreting and inducing secretion of appropriate cytokines from the T-helper cells. Depending on the nature of the T-helper cells (Th1 or Th2 type cells) this may lead to enhancement of the cell mediated response or antibody response by pertinent cytokines (e.g. γ-interferon (IFN) or interleukin (IL)-4, respectively) (Bais et al. 2005, Nguyen et al. 2005). Due to viral or environmental factors the Langerhans cells can be depleted from the site of HPV infection, which may impair/delay the immune response. Some researchers have observed a decrease in the number and functions of Langerhans cells in the epithelium of the cervix in women who smoked, suggesting that the decrease in the antigen presentation could contribute to the development of CIN (Szarewski et al 2001, Arcavi 2004). Smoking is known to induce cytokines Th1

and Th2 and may thus have an adverse effect on the immune response (Cozen et al. 2004, Whetzel et al. 2007, Nakaruma et al. 2008).

Infected epithelial cells may act as (non-professional) antigen presenting cells (Stanley 2008). Mostly endogeneous antigens expressed by the genome of the infecting virus are presented by the MHC class I molecules to the T-cell receptor. The non-professional antigen presenting cells may lack pertinent cell surface molecules (e.g. B7) that are needed for cell-cell signalling in conjunction of the antigen presentation, and proper stimulation of the cytotoxic T-cells (Doorbar 2007). Co-factors of cervical cancer (e.g. *C. trachomatis*) may induce downregulation of these cell-surface molecules by perturbating the local cytokine responses, and thus "paralyze" immune response to HPV (Whiteside 2008, Simonetti et al. 2009).

Most if not all MHC (HLA) class II molecules responsible for antigen presentation are capable of presenting peptides derived from the L1 protein of HPVs (Zehbe et al. 2005). This is indicated by no genetic defects associated with susceptibility to HPV infections, no heterozygosity advance with regard to persistent HPV infections or cervical neoplasia, and the fact that 100% of individuals develop high titre antibody responses to vaccination after HPV L1 VLP vaccines. On the other hand, there exists a genetic predisposition to persistent HPV infection or associated cervical neoplasia (Castro et al. 2007). Most likely, however, this is due to differences in cytotoxic T-cell responses to the early viral proteins expressed in HPV transforme cells – the identified MHC class II associations may be explained by linkage disequilibrium with the MHC class I (Liu et al. 2007).

Antibody responses

The study of antibody responses to HPV is a useful tool in understanding the natural history of HPV infection, the cancer association of HPV and for vaccine development. Antibodies against L1-containing virus-like-particles (VLPs) are HPV type-specific and become detectable 2 to 12 months after infection (Carter et al. 1996, Schiller and Hidesheim 2000). Antibodies are not responsible for clearance of the virus but are involved in protection against the infection (Wang and Hildesheim 2003, Nguyen et al. 2005). The major isotypes of the antibody response against HPV are (Immunoglobulin) IgG1 and IgA. Studies on immune response to HPV infection showed that serum IgG antibodies persist longer than IgA antibodies (Wang et al. 2000). On the other hand, IgA antibodies appear early and are markers for recent or active HPV infection, whereas IgG antibodies are stable markers of lifetime cumulative HPV exposure (Dillner etal. 1996; af Geijersstam et al. 1998, Onda et al. 2003).

Serum IgG against HPV16 is detected in 50 to 60% of women who are positive for HPV16 DNA (Kirnbauer et al. 1994, Le Cann et al. 1995, Carter et al. 1996, Kjellberg et al. 1999). Most women who seroconvert will do so 6–12 months after infection but 10–20% convert during the time HPV DNA is detectable (af Geijersstam et al. 1998, Andersson-Ellstrom et al. 1996). When investigating

both serum and cervical IgA in women with incident HPV infection, the authors observed that within 18 months of the first detection of HPV16, 87.3% of the women had developed anti-HPV16 IgA in cervical secretions (Onda et al. 2003).

Women who cleared their infection revert to seronegativity faster than women with persistent HPV16 infection (Nardelli-Haefliger et al. 2003). HPV-seropositivity has in a few studies been associated with CIN persistence and severity of the lesion. Anti-VLP-L1 HPV16 antibodies were detected in approximately 30% of LSIL patients, and in 50% of women with HSIL (Sasagawa et al. 1996, Bontkes et al. 1999, Wideroff et al. 1999). Thus, it is natural that longitudinal seroepidemiological studies disclosed that HPV16-seropositive women have an increased risk for subsequent development of CC (Lehtinen et al. 1996, Dillner et al. 1997, Lehtinen et al. 2001).

Antibodies against non-structural HPV proteins have also been investigated. Anti-E2 IgG was detected in 67% of HPV16 DNA-positive women (Rosales et al. 2001). Anti-E6 and E7 antibodies can be found in CC patients but are not useful as indicators of CC prognosis (Silins et al. 2002). Lehtinen et al. evaluated HPV16 and 18 E6 and E7 responses in samples taken 1–20 years before time of diagnosis. Antibodies were detected in only 7% of women who subsequently developed cancer (Lehtinen et al. 2003). Although IgG antibodies to the HPV16 E2 were found to be inversely associated with progression of CIN, increased IgA and IgG antibody levels to the HPV 18 peptide indicated a significantly increased risk of adenocarcinoma (Lehtinen et al. 1992), suggesting that there may be differences in the humoral immune response to the major oncogenic HPV types.

Cell-mediated immune response

Cellular immunity plays a determining role in the early phase of HPV infection and is also involved in the immune response to CC (Frazer 2009). Immunohistological studies of genital warts and animal models have shown that non-regressing warts and lesions are accompanied with very low infiltrates of T cells (CD4+ and CD8+) (van der Burg et al. 2007, Kim et al. 2008), langerhans cells (Guess and McCance 2005, Caberg et al. 2008), proinflammatory cytokines (Ashrafi et al. 2006, Frazer 2009), and that there is a lack of systemic T cell responses to HPV proteins characteristic of an adequate regression (Suprynowicz et al. 2008, Fazer 2009). Evidence shows that HrHPVs downregulate IFN-inducible gene expression and its signalling pathways (Um et al. 2002, Stanley 2008). The Th1-Th2 model of immunoregulation may play a role in the natural history of HPV infection. A switch from Th1 to Th2 type cytokines has been suggested in cervical carcinogenesis (Frazer 2004, Bais et al. 2005). Keratinocytes infected by HPV can modify the immune response through the production of Th2 cytokines in squamous carcinomas, configuring an evasion mechanism against the T-cell mediated immune response (Frazer 2009).

Experimental studies show that cigarette smoking decreases the secretion of proinflammatory cytokines such as IL-1 and IL6 (Arcavi 2004) and suppresses IL2 and IFNγ production (Ouyang et al. 2000, Hussain et al. 2008), suggesting the possibility that cigarette smoking interacts to increase cervical carcinogenesis development.

HPV immune evasion

HPV infections are slow to induce measurable immune responses or clear, suggesting that HPV may have developed methods to evade host immune mechanisms. The primary mechanism of viral immune evasion for HPV infection is likely avoidance of antigen presentation (Doorbar 2007, Kanodia et al. 2007, Patel and Chiplunkar 2009). A brief description of different mechanisms involved is presented in Figure 2.2.
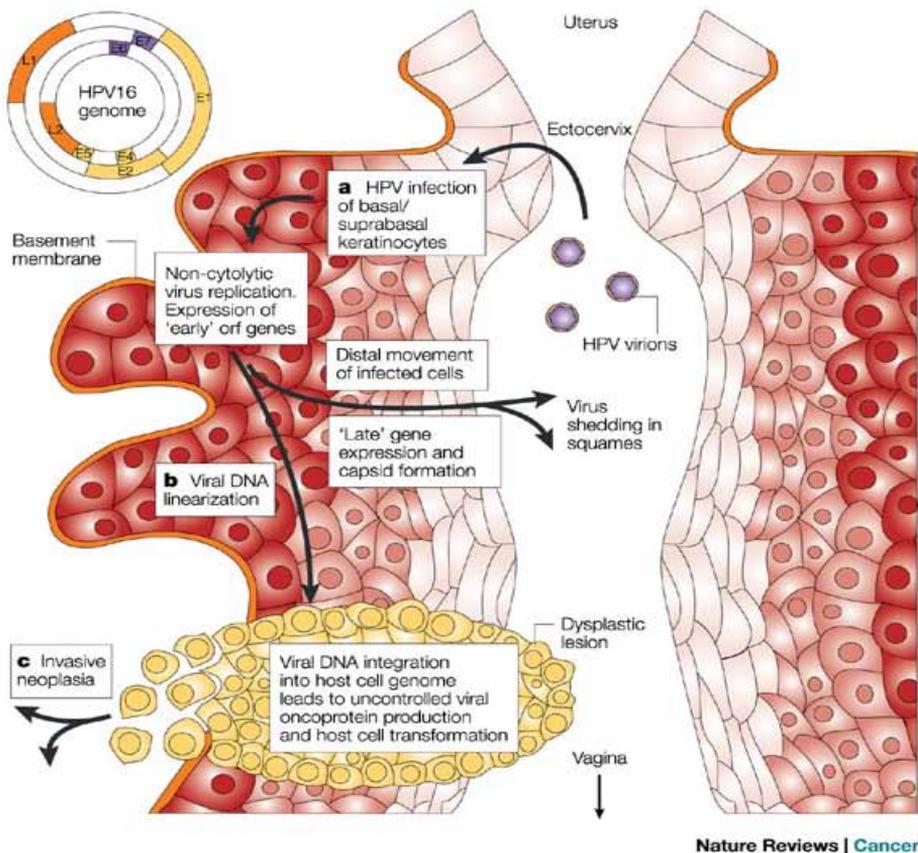

FIGURE 2.4 Human papillomavirus (HPV) immune evasion (Tindle 2002)

HPV infection and reproduction are totally dependent upon a complete programme of keratinocytes differentiation, expressing high levels of viral proteins only in the upper layers of the epithelia. Therefore HPV does not induce cytolysis or cytopathic death in its host cell but in the virus infected keratinocytes, which results in no alarm signal to the immune system and allows persistent and chronic infection (Tindle 2002, Kanodia et al. 2007).

Modes of transmission of HPV infection

HPV is essentially sexually transmitted (Svare et al. 1998; Centers for Disease Control and Prevention 2008). The mean incubation period is estimated to be 2 to 3 months, ranging from a few weeks to eight months (Handsfield et al. 1997). The HPV transmission probability per sexual partnership has been estimated to be 60% in mathematical modelling analyses (Barnabas et al. 2006). However, reports of high rates of incident, albeit transient, HPV infection in small children have raised the issue of alternative modes (mother to child) of acquiring the virus (Stevens-Simon et al. 2000, Sinclair et al. 2005, Cason and Mant 2005, Puranen et al. 1997, Rintala et al. 2005).

HPV detection

Until recently, the only means of diagnosis of HPV infection was direct or enhanced *Visual Inspection*, a specific but not particularly sensitive method (Denny et al. 2002, Sarian et al. 2005). The past decades have witnessed the introduction of increasingly effective molecular diagnostic techniques for HPV infection.

The *Southern Blot* method is the basis of all other specific detection methods. In this method, DNA is extracted and enzymatically digested. The resulting fragments are then separated by electrophoresis according to their size. The isolated fragments are denaturated and identified with the help of single-strand fragments of complementary DNA or RNA labelled with radioactive or calorimetric molecules. This requires relatively large quantities of DNA, is laborious and is impossible to automate (Cuzick et al. 1999).

The *Dot Blot* method (ViraPap, Viratype) was a simplified version of the Southern Blot method that avoids the enzymatic digestion and electrophoresis stages. It was partially automated but required large amounts of DNA and had a lower sensitivity and specificity (Cuzick et al. 1999).

The *filter in situ hybridisation* method was also derived from the Southern Blot but omitted the DNA extraction step. The target cells were applied directly to a solid support and treated to DNA denaturation and identification. While the method was simple, it did not perform adequately in clinical use (Demeter et al. 1987, Cuzick et al. 1999).

In situ hybridization (ISH) demonstrates nucleic acid sequences directly (in situ) in the cells. Cells or tissue sections are fixed on a slide and examined under a

light microscope enabling the assessment of DNA or RNA localization to be related to the morphologic aspects of the lesions. Identification of HPV18 in cervical adenoma tissue was confirmed with this methodology (Leminen et al. 1991). ISH with biotinylated HPV probes (Syrjänen et al. 1986) is a user friendly version of this method.

The *Hybrid Capture* Assay I (*HC-I*) was first introduced by Digene in 1995 (Clavel et al. 1999). HC-I was a relatively fast, liquid hybridization assay designed to detect 9 high-risk HPV genotypes (16, 18, 31, 33, 35, 45, 51, and 56) and one of 5 low-risk types (Cuzick et al. 1999). Initially, this was to be used to enhance the sensitivity of conventional Pap testing and to provide a meaningful negative predictive value for assessing cervical dysplasia. Since then, a second generation of Hybrid Capture assay (*HC II*) has been developed. The assay is a non-reactive, chemiluminescence that is easy to perform and can therefore be used in most clinical laboratories. The HC II is an effective test that requires less expertise than cytology (Terry et al. 2001). HC II detects more HPV genotypes and has high sensitivity and specificity (Hesselink et al. 2005, Brink et al. 2007, Kotianiemi-Talonen et al. 2008). The intra- and inter-laboratory agreement rates between these methods range from good to excellent, i.e. they are well reproducible (Schiffman et al. 2000, Gravitt et al. 1998, Castle et al. 2004, Kulmala et al. 2004, Carozzi et al. 2005).

The *Polymerase Chain Reaction* (PCR) is a selective target amplification assay capable of exponential and reproducible increase in the HPV sequences present in biological specimens (Brink et al. 2007). The amplification process can in theory produce one billion copies from a single double stranded DNA molecule after 30 cycles of amplification. The sensitivity and specificity of PCR-based methods do vary, depending on the DNA extraction procedures, site and type of clinical sample, sample transport and storage, primer sets, the size of the PCR product, reaction conditions and performance of the DNA polymerase used in the reaction, the spectrum of HPVDNA amplified and ability to detect multiple types (Brink et al. 2007). Most laboratories use PCR assays which utilize consensus primers including GP5+/6+, MY09/11, or SPF primer sets, directed to a conserved L1 gene, and hence able to detect all mucosal HPV types. Detection of the hybridized PCR product is done by a colorimetric reaction (Gravitt et al. 1998, Kleter et al. 1999) or by chemiluminescence (Van der Brule et al. 2002). Recently, a quantitative and high-throughput method was developed (Schmitt et al. 2006) based on Luminex suspension array technology. This method relies on detection of the consensus primer GP5+/6+ products with type-specific oligonucleotide probes coupled with fluorescence-labelled polystyrene beads, and allows detection of up to 100 different HPV types simultaneously. If one is interested in a particular HPV type, type-specific PCR can be applied. Confirmation of the specificity of type-specific PCRs, as with consensus primer PCRs, can be done by (regular or reverse) filter hybridization or by EIA, but also in real-time (Josefsson et al. 1999, Hesselink et al. 2005, Moberg et al. 2003). The possibility to sequence the amplicons has become more and more important in the identification of HPV subtypes (e.g. the African

vs. European subtypes of HPV16). Antibodies induced in natural infections by the different subtypes cross-react (Pastrana et al. 2004). A great advantage of real-time PCR assays is the possibility to quantify the HPV-DNA in the specimen. Several studies have shown that the amount of hrHPV present in a cervical smear (the "viral load") as measured by real-timePCR is predictive for the presence or development of high-grade cervical lesions (Josefsson et al. 2000, Ylitalo et al. 2000, Van Duin et al. 2002, Snijders et al. 2003, Snijders et al. 2006).

Detection of HPV-RNA will in the near future be applied to the distinction of transient HPV infections (or mere HPV DNA contamination) from clinically significant or persistent infections (Hafner et al. 2007, Khan and Singer, 2008).

HPV *serology* measures specific antibodies against different HPV types (Carter et al. 1996, Dillner et al. 1996). It is a way of detecting past infection and is very suitable for epidemiological studies because serum antibodies are stable over time (af Geijerstamm et al. 1998). Type-specific HPV serology has been important in the elucidation of the etiological role of HPV and CC (Lehtinen et al. 1996, Wang et al. 2000, Lehtinen et al. 2001). The most commonly used method is enzyme-linked-immunosorbent-assay (ELISA) using HPV virus-like particles (VLPs) as the antigen. The standard ELISA detects anti-HPV antibodies of the IgG or IgA isotopes. The sensitivity of serology for detecting anti-HPV16 antibodies is approximately 50% in best case scenarios, may be up to 65–75%, however, for HPV18 it is less than 50% (Kjellberg et al. 1999, Carter et al. 2000). As reported early by Kirnbauer and colleagues, IgG anti-HPV antibodies are found in only about 60% of women testing positive for cervical HPV DNA (Kinbauer et al. 1994). Anti-HPV antibody detection can therefore not be used as diagnostic tool on the individual level since all HPV infected women apparently do not seroconvert. HPV seropositivity is associated with the numbers of sexual partners and cytological lesions (Castle et al. 2002, Dillner et al. 1999).

HPV – a necessary cause of cervical cancer

The role of HPV in CC development has been studied in depth, culminating in the conclusion that the presence of certain HPV types is necessary for the development of CC (zur Hausen 2002, Roden and Wu 2006, Doorbar 2006, Walboomers et al. 1999, Bosch et al. 2002). Of the numerous HPV viruses, HPV16 is the type most commonly found in precancerous and cancerous lesions, followed by HPV18. In fact HPV16 and 18, along with 3 other hrHPV types (31, 33 and 45), are responsible for 85% of HPV infections that result in CC (Munoz et al. 2003). Other hrHPV types associated with CIN and CC are 35, 39, 45, 51, 52, 56, 58, 59 and 66 (with strong association) and 26, 68, 73 and 82 (with possible association) (zur Hausen 2006). Even though most sexually active women are exposed to HPV infection at least once in a lifetime and more than 50% of women worldwide will acquire a genital HPV infection (Gunell 2006), development of CC is, however, not common. Thus, HPV is not considered to be a sufficient cause of CC. A possible explanation is that

other cofactors are also necessary to trigger CC formation. This may be through alteration of the host (e.g. immune system), direct carcinogenic potential (creation of genotoxic adducts) or direct interaction with HPV in one way or another.

2.2.3.2 Other sexually transmitted diseases

Conflicting results on the role of herpes simplex virus type 2 (HSV-2) infection in cervical carcinoma and its precursors have been reported in several studies. HSV-2 may act in conjunction with amplified HPV infection to increase the risk of invasive cervical carcinoma (Hildesheim et al. 1991, Olsen et al. 1998, Smith et al. 2002). Evidence from longitudinal studies, however, indicates no role of HSV-2 in cervical carcinogenesis (Ferrera et al. 1997, Lehtinen et al. 2002).

Taking into account the central role of genital HPV infection, *C. trachomatis* is likely to be a risk factor of cervical squamous cell carcinoma (SCC) but its role has been difficult to prove (Koskela et al. 2000, Anttila et al. 2001, Wallin et al. 2002, Madeleine et al. 2007). Its effect may possibly modulate the host's immunity and/or precipitate chronic inflammation as persistence of oncogenic HPV infections is more likely among women with a previous *C. trachomatis* infection (Silins et al. 2005).

2.2.3.3 Age

Cancer of the cervix occurs most often in women over the age of 40. The rise in incidence of CC begins at ages 20–29, after which it increases rapidly reaching a peak between ages 45–49 in Europeans populations. In developing countries, the peak is often at a rather later (55–60) age (Curado et al. 2007, Stewart et al. 2003).

In Finland, the incidence of CC has increased especially in fertile-aged women during the first years, most likely due to an increase in HPV occurrence (Anttila et al. 1999, Laukkanen et al. 2003, Barnabas et al. 2006).

2.2.3.4 Tobacco

A positive correlation between the incidence of CC and other cancers known to be related to cigarette smoking across populations prompted the hypothesis that smoking may affect the risk of CC (Winkelstein 1977).

A review of some case-control (Stellman et al. 1980, Clarke et al. 1982, Brinton et al. 1986, Cuzick et al. 1996, Parazzini et al. 1998, Hirose et al. 1996) and cohort studies (Beral et al. 1988, Tverdal et al. 1993, Zondervan et al. 1996, Tulinius et al. 1997, McIntyre-Seltman et al. 2005, Tolstrup et al. 2006) shows that smokers have an increased risk of preinvasive neoplasms and invasive squamous-cell CC compared to non-smokers. In an earlier IARC evaluation of tobacco smoking, the

working group noted that the effect of smoking is confounded by sexual behaviour variables, but the data were not adequate to remove the confounding effect, and that a reasonable conclusion from the available studies of invasive CC is that the results, although they indicate a positive effect of smoking, are compatible with the residuals effects of variables that play a fundamental role in the aetiology of CC (IARC 1986). The extent to which the relationship between smoking and CC reflected a causal association independent of infection with HPV remained a cause of concern. It was believed that the association of smoking with CC may be causal, may even reflect causality via an effect of smoking on risk for HPV infection, or may reflect confounding or risk modification among women with HPV infection (IARC 1996).

In a few cohort studies (Deacon et al. 2000, Castle et al. 2002, McIntyre-Seltman et al. 2005, Mosciki et al. 2001) and case-control studies (Chichareon et al. 1998, Ngelangel et al. 1998, Munoz et al. 1993, Ho et al. 1998, Yoshikawa et al. 1999, Kjellberg et al. 2000, Gunnell et al. 2006) reporting on the association between smoking and CIN or ICC, the point estimates were adjusted for HPV DNA status. In these studies, the association between CC and smoking was not eliminated even though most studies additionally controlled for several well-established risk factors for CC. Women who smoked for a long period or who smoked heavily generally had the highest risk. In several of these studies, the relation was restricted to, or strongest among, recent or current smokers. The highest point estimates were observed among women who had started smoking early in life (La Vecchia et al. 1986, Daling et al. 1996, Plummer et al. 2003).

Several cohort studies (Deacon et al. 2000, McIntyre-Seltman et al. 2005, Tolstrup et al. 2006) and case-control studies (Bosch et al. 1992, Eluf-Neto et al. 1994, Chaouki et al. 1998, Olsen et al. 1998, Ho et al. 1998, Santos et al. 2001, Rolon et al. 2000, Castle et al. 2002, Shields et al. 2004, Rajeeran et al. 2005), including the IARC multicentre pooled analysis of 10 studies of invasive CC (Plummer et al. 2003), examined tobacco smoking as a co-factor to HPV infection by restricting the analysis to HPV DNA-positive study participants. The results from these analyses showed no significant alteration in risk whether or not the study participants were HPV DNA-positive. A nested case–control study of CIN 3 investigated the effect of smoking among HPV-seropositive cases and controls and found that the effect of smoking remained, and there was evidence of a dose-response relationship (Deacon et al. 2002).

No association between smoking and adenocarcinoma and adeno-squamous-cell carcinoma of the cervix has been observed (Brinton et al. 1986, Ursin et al. 1996, Chichareon et al. 1998, Ngelangel et al. 1998, Madeleine et al. 2001).

Tobacco chewing has rarely been studied as a risk factor of CC. To date, only one study has reported an association between tobacco chewing and increased risk of invasive CC in Southern India (OR = 4.0, 95% CI 1.2–13.3) (Rajkumar et al. 2007).

Smoking has been associated with persistent HPV infections (Szarewski et al. 2001) and decreased clearance of CIN lesions (Szarewski et al. 1996). Reduction in lesion size was highly correlated both with the reduction in reported amount of smoking and with the final-visit cotinine measurements (Szarewski et al. 1996).

Whether the link between smoking, HPV and CC is related to: 1) Genotoxic adducts of tobacco in the cervix epithelium (Ali et al. 1994, Phillips et al. 1994, Prokopczyk et al. 1997), 2) Effects on HPV transformation (Motoyama et al. 2004), or 3) Localised immunosuppression (Poppe et al. 1995), has been discussed. Tobacco-specific carcinogens and polycyclic aromatic hydrocarbons have been identified in the cervical mucus or epithelium of smokers (Melikian et al. 1999, Prokopczyk et al. 1997). These compounds can damage cellular DNA and may cooperate with HPV to produce malignant transformation (Simons et al. 1995). Finally there is also evidence that some studies have tried to show the effects of the harmful substances absorbed by the human body during the act of smoking in the immunological defence of the cervical epithelium. Current smokers have significantly lower Langherlans cell counts than nonsmokers in normal cervical epithelium and in CIN, HPV infection, or both (Poppe et al. 1995).

2.2.3.5 Immune compromization

Women infected with the human immunodeficiency virus (HIV), the virus that causes AIDS, or who have used medication that suppresses the immune system have an excess risk of developing CC. CC is one of the "AIDS-indicative diseases". Higher risk of HPV infection and lower HPV clearance have been observed in women infected with HIV than in uninfected women (Palefsky et al. 1999, Ho et al. 1998, Rowhani-Rahbar et al. 2007). Furthermore, the high prevalence, incidence and persistence/progression of SILs appear to be associated primarily with increased HPV persistence that may result from immunosuppression related to HIV infection (Hawes et al. 2006, Six et al. 1998).

Long-term immunosuppression seems to increase the risk of CC among other anogenital cancers: in a few population-based follow-up studies, the observed incidence of cervical cancer among renal transplant patients has been higher than the expected rate (IARC 2006). In a study conducted in Australia and New Zealand, the age-standardised incidence ratio in transplant recipients was 3.3 after a mean follow-up of 5.8 years, whereas, it was 0.74 for dialysis patiens (Fairley et al. 1994). In a register-based study from the Nordic countries, the standardised incidence ratio for cervical cancer in transplant recipients was increased after an average of 4.8 years of follow-up (Birkeland et al. 1995). The increase was most notable in fertile-aged women among whom impaired immune system probably allows persistent HPV infection.

2.2.3.6 Other risk factors

There is evidence that numerous other risk factors such as *irregular or total lack of screening* (Bosch et al. 1992, Leyden et al. 2005, Nygard et al. 2002), *number of sexual partners* (Biswas et al. 1997, Kjaer et al. 1992, Agarwal et al. 1993, Bosch et al. 1996, Castellague et al. 2002, Franceschi et al. 2003, Hammouda et al. 2005), *long term oral contraceptive use* (Appleby et al. 2007, Smith et al. 2003), m*ultiparity* (Autier et al. 1996, Franceschi et al. 2003, Hammouda et al. 2005, Munoz et al. 2002), *early age of sexual debut* (Biswas et al. 1997, Bosch et al. 1992, Ferrera et al. 2000), *low socio-economic status and ethnicity* (de Sanjose et al. 1997, Ries et al. 2008), *micronutrients deficiency* (Garcia-Closas et al. 2005, Potischman et al. 1996), g*enetic susceptibility* (Zelmanowicz et al. 2005, Magnusson et al. 1999) may increase the risk of CC. Highest risk estimates were observed among women with HPV infection.

2.2.4 Prevention

2.2.4.1 Screening and HPV testing

The objective of cervical screening is to prevent invasive CC by detecting and treating women with high-grade SIL i.e CIN 2 and 3 lesions.

Conventional cytology (Pap smear) screening entails collection of cervico-vaginal epithelial cells using a wooden spatula or a brush, preparation and fixation of the smear. Screening with conventional cytology has resulted in a marked decrease in cervical cancer incidence and mortality in a number of countries. However, in some countries the effect of screening has been virtually nonexistent (International Agency for Research on Cancer 2005). In addition to the lack of organisation, the blame has often been laid on the quality of conventional cytology. Worldwide, the variation in the accuracy of conventional cytology is wide: estimates for the sensitivity for CIN 2+ vary from about 30% to 90% and, respectively, for the specificity from 85% to nearly 100% (Soost et al. 1991, Nanda et al. 2000, IARC 2005, Arbyn et al. 2008c). Reproducibility of conventional cytology measured through intra and interobserver variability has been at best moderate to good (Branca et al. 1996, Cocchi et al. 1997, Branca et al. 1998, Woodhouse et al. 1999, Gupta et al. 2001, Chhieng et al. 2002).

Organised cytology screening with systematic call, recall, follow-up and surveillance systems has shown the greatest effect (e.g. Finland) (Nieminen et al. 2004; Kotaniemi-Talonen et al. 2007). Failure and difficulties in organizing Pap smear screening in low- and medium-resourced countries have prompted the search for and evaluation of alternative screening test such as visual screening after the application of acetic acid (VIA) or Lugol's iodine (VILI) and HPV DNA testing (IARC 2004, Sankaranarayanan et al. 2005a).

Liquid-based cytology (LBC) is a modification of conventional cytology that is widely used for primary CC screening. With this technology cervical samples are collected into liquid solution, which is then used for cytological slide preparation. The residual material may be used for additional testing, e.g. HPV DNA detection. Several LBC tests are commercially available, among which ThinPrep (by Cytyc Corporation) and the BD SurePath System (formerly AutoCyte PREP System, by BD Diagnostics, Diagnostic Systems – TriPath) are the best-known and most studied (Arbyn et al. 2008a).

Recent randomised studies and meta-analyses have shown that the performance of LBC in terms of CIN 2+ detection is comparable to conventional cytology (Davey et al. 2006, Ronco et al. 2007, Arbyn et al. 2008b). However, some studies have reported a smaller number of unsatisfactory smears with LBC than with conventional cytology, and the time needed for interpretation is possibly shorter with LBC (Colgan et al. 2004, Dowie et al. 2006, Doyle et al. 2006, Ronco et al. 2007).

The fact that cervical neoplasia are caused by persistent infection with oncogenic types of HPV has led to the evaluation of **HPV testing** as a primary screening and/or secondary test for cervical neoplasia. The optimal age for HPV testing is likely to be between 30-40 years i.e. before the risk of CC starts to increase. HPV testing is the most objective and reproducible of all currently available cervical screening tests. The sensitivity of HPV testing in detecting CIN 2 and 3 lesions varied from 66 to 100% and the specificity varied from 62 to 96% in several cross-sectional studies (Franco 2003, Koliopoulos et al. 2007, Sankaranarayanan et al. 2005). In randomized trials, the sensitivity of HPV test for the detection of CIN is greater than that of Pap testing (Kotaniemi-Talonen et al. 2005, Bulkmans et al. 2007, Mayrand et al. 2007, Naucler et al. 2007, Sankaranarayanan et. al. 2009). A pooled data from seven European perspective screening studies shows that the cumulative incidence of CIN3/cancer was 0.27% among women negative for HPV DNA, whereas the respective cumulative incidence among women with negative cytological test was significantly higher, 0.97% (Dillner et al. 2008).

Self-collected samples for testing of oncogenic HPV is a potentially viable screening option that holds promise for women in under-resourced areas or those who are reluctant to participate in screening programmes, save for the limited evidence supporting it (Stewart et al. 2007).

2.2.4.2 Vaccination

While early detection of asymptomatic precancerous lesions by screening and their effective treatment leads to the prevention of ICC and premature death, the fact that CC is caused by persistent infection by one or more of the 15 oncogenic HPV types, with HPV types 16 and 18 causing more than 70% of cervical cancers, provides the exciting opportunity for prevention through vaccination.

Vaccinations with virus VLP were first found to be safe and highly protective in animal models. Rabbits that were immunized with cotton-tail rabbit papillomavirus (CRPV) VLPs were protected upon CRPV challenge and neutralizing antibodies were detected in most of these studies (Breitburd et al. 1995, Jansen et al. 1995).

Currently, two vaccines against human papillomavirus infection are commercially available, bivalent Cervarix™ (by GlaxoSmithKline) and quadrivalent Gardasil™ (by Merck and Co. Incorporation). Both vaccines are made from L1 VLPs i.e. empty protein shells resembling the real virus, for which they are non-infectious. Both vaccines are targeted against HPV types 16 and 18 that are estimated to cause altogether 70% of cervical cancers worldwide (Muñoz et al. 2004). In addition, Gardasil additionally targets two low-risk HPV types, HPV6 and 11 that cause 90% of genital warts.

VLP vaccines to prevent papillomavirus infections in humans have been evaluated in several studies (phase I to phase III) (Harper et al. 2006, Koutsky et al. 2002, Koutsky and harper 2006, Villa et al. 2006, The Future II Study Group 2007, Paavonen et al. 2007). The results from these studies indicate that HPV VLP vaccines are highly immunogenic in early adolescents (aged 9–13) and young women. The antibody titres are 2-fold higher among prepubertant boys and girls than among young women aged 16–26 years (Villa et al. 2005, Pedersen et al. 2007, Reisinger et al. 2007, Block et al. 2007, Dawar et al. 2007, Petäjä et al. 2009). This is in concordance with high immuno-response of other vaccines in children (Reisinger et al. 2007). In phase II clinical trial, quadrivalent HPV6, 11, 16 and 18 L1 VLP-induced antibody titres reach their highest level 7 months following the first vaccine dose. The titres then decline, reaching a plateau 18–24 months later. This plateau is maintained for at least 5 years, with 5-year levels that are similar to the titres naturally induced by HPV types 6 and 18 and that are higher than the titres naturally induced by HPV types 11 and 16 (Villa et al. 2006). At 24-month follow-up, over 96% of participants in the HPV vaccine group were seropositive for HPV types 6, 11 and 16; however, only 68% of participants were seropositive for HPV type 18. The significance of this reduction remains unclear as immune memory is induced by the vaccine (The Future II Study Group 2007). This has not been noted with the AS04-adjuvanted bivalent HPV16/18 vaccine (Harper et al. 2006). Geometric titers (GMTs) for vaccine induced antibodies against HPV16 or HPV18 infections were substantially higher ($\geq$11-fold) than those seen in natural HPV16 or HPV18 infections at all timepoints over 5.5 years (Harper et al. 2006). High seroconversion rates and strong vaccine-induced antibody response were also evident in the interim (14.8-month) results of the papilloma trial against cancer in young adults (PATRICIA) study, a large phase III trial of the AS04-adjuvanted HPV16/18 vaccine. At month 7, peak GMTs were 313-fold (HPV16) and 211 (HPV18) higher than corresponding values in women who had cleared natural HPV16/18 infections (Paavonen et al. 2007).

The results from vaccine efficacy (phase III) trials have shown high efficacy against HPV 16 and 18-related CIN 2/3 and adenocarcinoma *in situ* lesions among

adolescents and young women naïve for these HPV types and received at least one dose (of the three recommended) of the vaccine. The quadrivalent vaccine prevented 100% of HPV16 and HPV18-related cases of high grade (grade 2/3) vulvar or vaginal neoplasia, and 95% of HPV6, 11, 16, 18-related cases of CIN (grade 1, 2, 3) (The Future II Study Group 2007). The AS04-adjuvanted HPV16/18 vaccine demonstrated 90%–100% efficacy in preventing CIN 2+ and 89% efficacy in preventing CIN 1+ associated with HPV16/18 infections (Paavonen et al. 2007, Paavonen et al. 2009). Results from the phase II trial showed that the HPV16/18 vaccine efficacy was 100% in terms of preventing HPV16/18-associated CIN 1+ or CIN 2+ after at least 5.5 years of follow-up (Harper and Dubin 2007) .

HPV vaccination holds great promises for CC prevention, but there are still several issues that need to be resolved before it can be widely implemented in high-risk developing countries (Agosti et al. 2007). These include: current high costs of the vaccine, affordability, feasibility, acceptability, logistics of vaccine delivery (in view of the needs for three doses spread over 6 months, improved strategies and vaccine platforms to reach out to pre- or early-adolescent girls), long–term immunogenicity and efficacy in preventing cervical neoplasia, cross-protection against HPV types not targeted by the vaccine antigens and the efficacy of different, more logistically feasible dose regimes in including and maintaining immunogenicity and long term protection against cervical neoplasia. To initiate HPV vaccination in low- and medium-resource countries, vaccination costs should be dramatically reduced both by lowering the costs of the vaccine and of vaccine delivery.

A population-based phase III study in Finland will consider the long-term outcomes in HPV16/18-vaccinated adolescents versus those receiving a control vaccine or no HPV vaccine at all (Lehtinen et al. 2006). While prophylactic vaccination is likely to provide important future health gains if vaccination is offered to girls before onset of sexual activity, cervical screening should still be continued for women, as the risk of being already infected with the oncogenic HPV types remains. For the vaccinated birth cohorts the mode of screening most likely will change in years to come.

3 AIMS OF THE STUDY

The general purpose of this work is to contribute to understanding the role of smoking in the natural history of HPV infection and its progression to CC.

The specific aims were:

1. To ascertain whether the association between smoking and CC is due to an independent role of smoking or due to residual confounding by oncogenic HPV types.

2. To compare the association between tobacco chewing in Côte d'Ivoire, West Africa and tobacco smoking in Finland, Northern Europe, and the age-stratified risk of multiple HPV infections and cervical SIL.

3. To evaluate the role of smoking in the impairment of the immune response following natural HPV16 and HPV18 infection.

4. To evaluate the role of smoking in immune response following immunization with adjuvanted HPV16/18-AS04 VLP vaccine.

4 MATERIALS AND METHODS

4.1 Data sources and study participants

4.1.1 The Nordic population-based biobanks (Paper I)

4.1.1.1 Characteristics of the serum banks

Five population-based serum banks collaborated in this nested case-control study: the Finnish Maternity Cohort (FMC), the Iceland Maternity Cohort (IMC), the Northern Sweden Maternity Cohort (NSMC), the Northern Sweden Health and Disease Study (NSHDS) and the JANUS Project in Norway.

The FMC was established in 1983. By 2006 it comprised around 1,300,000 serum samples collected from 750,000 women, practically all pregnant women attending maternity clinics during the first trimester of pregnancy. The samples are stored at -25˚C at the National Institute for Health and Welfare laboratories in Oulu, Finland (Pukkala et al. 2007).

The nation-wide IMC contained 96,000 serum samples, by 2003, collected from 50,000 women at 12 to 14 weeks of pregnancy for rubella screening. The samples have been stored since 1980 at -20˚C in the Department of Medical Virology, Landspitali University Hospital, Reykjavik, Iceland (Pukkala et al. 2007).

The NSMC consists of sera collected since 1975 from pregnant women screened for rubella immunity during week 14 of pregnancy in Västerbotten county and especially in the 1980s for some of the adjacent counties in Northern Sweden. So far, almost 120, 000 samples from 86,000 women have been stored at -20˚C at the virus laboratory of Umeå University, Sweden (Pukkala et al. 2007).

The NSHDS contains the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) cohort and the Västerbotten Intervention Program (VIP). The MONICA project contains material from population-based screenings for cardiovascular diseases carried out in 1986, 1990, 1994, 1999 and 2004. There are 14,000 sampling occasions of 9,000 individuals, 50% of whom are included in VIP. Established in 1985, the VIP is a long-term project intended for health promotion and including 74,000 individuals by 2004, of whom 70,000 had donated blood samples. The samples are collected annually from all residents aged 40, 50 and 60 years of age, in the Västerbotten county, northern Sweden, and stored as plasma at -80˚C at the University Hospital of Umeå, Sweden (Pukkala et al. 2007, Tedeschi et al. 2006).

The JANUS serum bank was established in Oslo, Norway in 1973 to identify early changes in chronic disease development. In 2003 the JANUS serum bank had 430,000 serum samples from 331,801 donors, 10% of them are Red Cross blood donors. About 145,000 female donors have been recruited during routine health

examinations in subsequent phases. Serum samples in 1974–1978 and in 1983–1991, are being stored at -25˚C (Pukkala et al. 2007).

4.1.1.2 Population-based cancer registries

The cancer registries of Finland, Iceland and Norway and the regional cancer registry in Umeå, Sweden are all population-based and country-wide (four northernmost Swedish counties for Umeå). Audits have shown that virtually all cases of cancer are being reported to the cancer registries by physicians and pathology/hematology laboratories, and 95% of them are histologically confirmed (Engeland et al. 1995). Following approval of the appropriate ethics committees, serum banks were linked to the national cancer registries using the unique personal identification number (PIN) available in the Nordic countries for each citizen.

4.1.1.3 Identification of cases and controls

To elaborate the association of smoking and CC, we performed two consecutive nested case-control studies (Studies I and II, based on 171 and 588 invasive CC cases matched respectively to 496 and 2861 unaffected controls). Study I had limited power to distinguish whether smoking was an independent co-factor in cervical carcinogenesis and in Study II, we assembled almost four times as large independent material. Most of cases in Study I were included in Study II (Table 4.1).

Cases were women diagnosed with invasive CC following their enrolment into one of the Nordic cohorts. Cases were identified by linking the serum bank data files with the national cancer registries. When serial blood samples were available, the first sample collected with the longest lag time to cancer diagnosis was chosen.

Controls were selected from the same serum bank as cases (Table 4.1). For each case, five female controls who were alive and free of cancer at the time of the case's diagnosis were randomly selected and matched for age at serum sampling (± 2 years), storage time (± 2 months) and for county in Norway and Sweden. If five controls could not be found, matching criteria of age at blood collection and length of frozen storage were widened.

The average lag times from serum sampling to cancer diagnosis was 4.7 years in Study I and 9.6 years in Study II. In Study I, the mean ages at diagnosis were as follows, the FMC: 34 years, the JANUS health examinations: 47 years, Norway (1974–1978): 46 years, Norway (1981–1992): 47 years and the NSHDS: 50 years. In Study II, the mean ages at diagnosis were as follows, the FMC: 38 years, the JANUS health examinations: 49 years, Norway (1974–1978): 56 years, Norway (1981–1992): 47 years, the JANUS Red Cross blood donors: 44 years, the NSMC: 37 years and the IMC: 34 years.

TABLE 4.1 Characteristics of the Nordic cohort for a nested case-control study of the cervical carcinoma risk associated with previous exposure to tobacco smoke.

Serum bank	Geographic location	Period of serum sampling	Female serum sample donors (no.) by the end of year 2003	Cases (no. in Study I/ Study II)	Controls (no. in Study I/Study II)	Age at diagnosis (years) in Study I/Study II
Janus Project	Three counties in Norway Several counties in Norway Red Cross blood donors in capital	1974–1978 1981–1992 1973–1991, 1997–2000	29,000 115,000 14,000	79/36 47/129 –/45	237/178 141/639 –/223	26–63/32–70 35–63/29–67 –/29–69
Northern Sweden Health and Disease Study (VIP & MONICA)	Two northernmost counties in Sweden	1985–present	38,000	4/–	12/–	39–60/–
Northern Sweden Maternity Cohort	Four northernmost counties in Sweden	1975–present	86,000	–/111	–/530	–/21–57
Finnish Maternity Cohort	Finland	1983– present	681,000	41/167	106/798	22–49/22–54
Icelandic Maternity Cohort	Iceland	1980– present	49,000	–/100	–/493	–/23–55
Total	Nordic countries	1973– present	1,008,000	171/588	496/2861	22–63/21–70

4.1.2 The DYSCER – Côte d'Ivoire study (Papers II)

The disease screening research study (DYSCER) was conducted from April 1995 to February 1996. The study took place in three outpatient gynaecology clinics of Abidjan, Côte d'Ivoire. Women attending the clinics were eligible for the study if they fulfilled the following inclusion criteria: age between 20 and 50 years, absence of history of lower genital tract neoplasia, and absence of active pregnancy. A questionnaire was administered to each participant to obtain information on tobacco use. The subjects underwent pelvic examination and the cervical Pap smears obtained were independently read twice, in Abidjan and in France, in order to minimize false negative/positive results. Colposcopy with biopsies was performed on women with HSIL. Cases included all women with cytological cervical SIL. Controls were chosen at random during the same time and in the same clinics among the women without cytological cervical lesions with a frequency matching for age (by decade) and center. A complete description of this material has been published previously (La Ruche et al. 1998).

4.1.3 The KUOPIO Cohort (Paper II, III)

A prospective follow-up study of 532 women (mean age 28.7 years, range 15 to 69 years) with cervical HPV infection was conducted at the Kuopio University Hospital, Kuopio, Finland between 1981 and 1992. The original aim was to establish prognostic factors associated with the clinical course of HPV infections identified in young women by cytology. The cohort participants were recruited among women whose routine Pap smear showed unequivocal changes of HPV infection, i.e., koilocytotic cells with or without dyskaryotic abnormalities. The participants were subjected to a thorough gynecological examination including colposcopy, repeat Pap smear, and directed punch biopsy (when clinically indicated). A questionnaire was also administered to each woman to obtain information on medical history and smoking habits. After the first visit, all the participants were scheduled to attend the gynecological outpatient clinic of the Kuopio University Hospital for follow-up visits at 6-month intervals. Cervical smears and serum samples were collected at each visit. A complete description of this material has been published previously (Kataja et al. 1992).

The comparative study (Paper II) included 2162 women (mean age 29.4 ± 7.1 years, range 20–50 years) from Côte d'Ivoire and 419 women (mean age 27.8 ± 7.9 years, range 15–49 years) from Finland with complete baseline data on HPV status, tobacco use, Chlamydia trachomatis infection and HIV status.

In Paper III, we collected data from the Kuopio Cohort. A total of 191 participants (mean age 27.5 ± 6.9 years, range 16-49 years) who had available HPV DNA biopsy data at the end of the follow-up, and for whom at least two serum samples (first and last samples of the follow-up) were available for serology and cotinine analyses, were included in the analyses.

These study protocols were each approved by the respective ethics committee in Côte d'Ivoire, France and Finland.

4.1.4 The PATRICIA Study (Paper IV)

Enrolment for the papilloma trial against cancer in young adults (PATRICIA) study took place from April 2004 to May 2005 in Finland. Healthy women aged 16–17 years were eligible to participate in the Finnish arm of this study with no exclusion criteria with regard to lifetime sexual partners before study enrolment. Individuals with intact cervix and agreeing to adequate contraception (barrier methods in combination with a spermicide or hormonal contraception) over the vaccination period were eligible for inclusion. Exclusion criteria were limited to women with a history of colposcopy, who were pregnant, or breastfeeding, or who had chronic or autoimmune disease or immunodeficiency.

Informed consent was obtained from each participant to the performance of any study procedures. All recruitment materials, informed consent/assent forms, protocols and amendments were approved by the Finnish National Ethical Review Committee. This study is registered with the European Clinical Trials Database.

This was a phase III double-blind, randomized controlled trial. In Finland, a total of 4,808 participants were randomised in a 1:1 fashion with an internet-based centralized randomisation system received either the AS04-adjuvanted HPV16/18 vaccine (GlaxoSmithKline Biologicals, Rixensart, Belgium) or, a control hepatitis A vaccine (GSK Biologicals), to provide a health benefit for all participants and ensure double-blinding. Allocation of treatment numbers was stratified by study site and by age. Because the study is gradually ending, a random sample of 216 study participants with individual vaccine allocation unblinded, wase selected from the three biggest Finnish study sites: the Finnish Family Federation, Helsinki, the University of Helsinki, Helsinki and the University of Tampere, Tampere for this study.

Each dose of HPV-16/18 L1 VLP AS04-adjuvanted candidate vaccine (Cervarix™) contained 20 mg each of HPV-16 and -18 L1 proteins self-assembled as VLP and adjuvanted with AS04 (50 mg 3-O-desacyl-40-monophosphoryl lipid A [MPL] and 500 mg aluminum hydroxide). The vaccine was produced using a Baculovirus Expression Vector System in which each type of VLP antigen was produced on a Hi-5 cell line derived from *Trichoplusia ni*. Each dose of the control hepatitis A vaccine contained 720 ELISA units (EU) of inactivated hepatitis A antigen and 0·5 mg aluminium hydroxide. The vaccines were supplied under double-blinding in identical 0·5 ml prefilled syringes and administered into the deltoid muscle on a 0, 1, and 6-month schedule. According to protocol the code was broken for the subset selected for this study in March 2009.

Blood samples were collected at baseline and at month 7 post-vaccination, and stored at the National Institute for Health and Welfare laboratories in Oulu,

Finland from each participant before the first vaccination and at month 7 to assess vaccine-induced immune responses to HPV16, HPV18 or related factors.

Participants completed and returned safety diary cards documenting symptoms experienced during the first 7 days after vaccination and symptoms within the first 30 days after vaccination as previously described (Paavonen et al. 2007).

4.2   Laboratory methods

For all the studies, the laboratory analyses were performed on coded specimens, with case-control status masked. The samples of a case and her matched controls were pipetted on the same microplate.

4.2.1 Cotinine detection (Papers I to IV)

Blood samples were measured for cotinine using a qualitative immunoassay method (OraSure Technologies, Bethlehem, Pennsylvania) that is carried out as a quantitative assay and is based on the competition between free cotinine in the sample and cotinine bound to horseradish peroxidase-labelled cotinine. Cotinine concentration was quantified by measuring the light absorbance of wavelengths of 450 nm and 630 nm and by comparing the cotinine concentration of each sample with the standard curve. This assay has a sensitivity of 95%–97% and a specificity of 99%–100% (Murray et al. 1993, Parish et al. 1995, Pérez-Stable et al. 1995). Careful testing of this method has revealed excellent correlations with established gas chromatography (Feyerabend et al. 1990) and radioimmunoassay (Knight et al. 1985). Regression dilution bias was assessed by measuring paired samples repeatedly with the same batch from the assay kit (Clarke et al. 1999).

4.2.2 HPV serology and detection (Papers I to IV)

The presence of IgA (Paper III) and G (Paper I, III, IV) antibodies specific for HPV types 16 and 18 was determined by means of a standard enzyme-linked immunosorbent assay (ELISA) as reported elsewhere (Dillner et al. 1996; Lehtinen et al. 2006). For the IgA assay, goat anti-human IgA HRP conjugate antibody (Novus Biologicals, Colorado, USA) was used for detection. IgG analyses were done applying VLPs kindly provided GSK Biologicals.

We employed baculovirus-expressed capsids comprising both the L1 and L2 proteins, with disrupted capsids of bovine papillomavirus used as the negative control. The specificity of HPV serology was found to be high, since no antibodies could be found in serum samples from virginal women and seropositivity had

a linear relation to lifetime number of sexual partners (Dillner et al. 1997). The sensitivity of HPV serology for detecting past HPV infection has been estimated to vary between 50% and 70% (Carter et al. 1996, Kjellberg et al. 1999). Seropositivity was defined as a titer greater than or equal to the assay threshold for HPV16 and HPV18. Dilutions 1/30, 1/300 and 1/3000 were used to identify the linear part of the absorbance reactions for expression of the results as OD values.

HPV DNA was detected using PCR (Côte d'Ivoire) (Paper II) and ISH (Finland) (Papers II and III) techniques. In Côte d'Ivoire, cervical specimen for HPV detection were collected with a plastic brush (Viba-Brush, CML, Nemours, France), placed in a transport medium (sterile RPMI 1640 medium supplemented with penicillin and fungizone), and frozen at -80˚C until testing. The presence of cervical HPV was tested in 547 participants including 224 women with cervical SIL (151 LSIL, 60 HSIL, and 13 cervical cancer cases) and 323 controls. HPV DNA was detected with a polymerase chain reaction (PCR)-based method using HPV L1 consensus primers (MY11 and MY09) affording the detection of a broad spectrum of genital HPV types as previously described (La Ruche et al. 1998).

For HPV DNA-positive samples, restriction fragment length polymorphism analysis of the amplimers produced during PCR was used to determine HPV types 6, 11, 13, 16, 18, 31, 33, 34, 35, 39, 40, 42, 44, 45, 52, 53, 56, 58, 59, 61, 62, 64, 66, 67, 68, 70, 71 (CP8061), 81 (CP8304), 82 (MM4 et IS39), 83 (MM7), 84 (MM8). Only samples that gave a strong enough signal to be detected clearly on gels after enzymatic digestion were considered for HPV typing. In Finland, formalin-fixed, paraffin-embedded biopsies from all patients were analysed using DNA-ISH technique with S- and biotinylated HPV DNA probes as described earlier (Syrjänen and Syrjänen 1986). Specific DNA probes for HPV types 6, 11, 16, 18, 31, and 33 were used. The presence of HPV DNA sequences in the lesions was indicated by dense condensations of black–silver grains, usually superimposed on the nuclei of the intermediate and superficial cells, in HPV lesions.

4.2.3 *Chlamydia trachomatis* serology (Papers I, II and III)

The presence of IgG antibodies specific for *C. trachomatis* was determined by the microimmunofluorescence (Hanna et al. 1980) (Paper I) and ELISA (Dillner et al. 1999) (Paper II, III) methods according to the manufacturer's instructions.

4.2.4 HSV-2 serology (Paper I)

The presence of IgG antibodies HSV-2 was also determined using a commercially available HSV-2 glycoprotein gG-2-based ELISA (Biokit SA, Barcelona, Spain) according to the manufacturer's recommendation (Lehtinen et al. 2002).

4.2.5 HIV (Paper II)

All sera from Ivorian women were screened in Abidjan by ELISA (Genelavia Mixte, Sanofi Diagnostics Pasteur, Paris, France). In Finland, there were no HIV-positive women diagnosed in the catchment area of Kuopio University Hospital between 1981 and 1986 (National Institute for Health and Welfare, Finland 2007).

4.3   Statistical methods

Paper I

The cotinine level that is accepted as defining an active smoker depends on the prevalence of smoking in the population (Murray et al. 1993, Cummings and Richards 1988). In this paper, the measured cotinine levels were categorized into 3 groups: less than 20 ng/mL for nonsmokers or persons passively exposed to tobacco smoke and 2 other categories of 20-<100 ng/mL and ≥100 ng/mL, corresponding approximately to average levels found among light and heavy smokers.

Relative risks, expressed as odds ratios (ORs), were estimated using conditional logistic regression for matched case-control sets of cervical cancer by means of GLIM4 (Numerical Algorithms Group, Oxford, United Kingdom) (Breslow and Day, 1980). The 95% confidence intervals (CIs) for the ORs were based on profile likelihood (Nelder, 1990). Heterogeneity in the point estimates was assessed with a likelihood ratio test. The nested models compared were one for overall effects of cotinine groups (20-<100ng/ml and ≥100 ng/ml) and the other one also including serum bank-specific effects of the cotinine groups. Unconditional logistic regression was applied to HPV16/18-seropositive cases and controls, including the matching variables (serum bank, subcohort, storage time and age at serum sampling) in the model.

Increasing tobacco smoke exposure was defined by blood cotinine levels. The cotinine levels for evaluating the relationship between cotinine dose and risk of invasive CC in Study II were quartiles among control women with cotinine level >20ng/ml and the cut-off level for heavy smoking which was close to the median, 102ng/ml. In the dose-response analyses the quartiles (68, 100, and 140ng/ml) of active smokers (>20ng/ml) were used. The dose-response relationship was tested by the likelihood ratio test by adding the numerical dose-response variable to the threshold model in which a variable indicated those, whose cotinine level was at least 20ng/ml. Likelihood ratio tests for linear trends in the logarithms of ORs were performed for increasing age at diagnosis among women with cotinine level 20-<100ng/ml and 100ng/ml or more with and without adjustment for HPV16/18, HSV-2 and *C. trachomatis*.

Paper II

In this study, different cut off levels were tested, and the optimum value, cotinine level of 20 ng/ml, was used as the cut-off. Descriptive statistics were used to determine the distribution of women according to cervical SIL, age and tobacco exposure, and HPV positivity. Frequency tables for tobacco use (smoking and/or chewing) among women stratified by age were produced. The effect of tobacco smoking and chewing on the risk of LSIL, multiple HPV infections and HSIL was estimated by ORs with 95% CI, derived from the logistic regression analysis. Because very few Ivorian women reported smoking evaluation for this habit alone in the logistic regression analysis was restricted to Finnish women. We conducted stratified analyses according to age, and the ORs were adjusted for C. *trachomatis* and HIV seropositivity (for Ivorian women). The level of statistical significance was p<0.05. All the statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, USA).

Paper III

Descriptive statistics were used to analyse the distribution of HPV16/18 antibody isotypes at baseline. McNemar's test was used to determine the proportion of change over time in HPV serostatus (dichotomous outcome variable) to take into account the fact that the observed proportions are not independent. Seroconversion and persistence of HPV16/18 antibodies were assessed. Logistic regression models were fitted and the relative risks (estimated as OR and 95% CI) calculated using SPSS13.0 (SPSS Inc., Chicago, USA). The model estimated the odds of seroconversion and the odds of maintaining persistent antibody response for HPV16/18 smokers, and used individuals with cotinine level less than 20 ng/ml, as the reference category. The ORs were adjusted for chlamydia seropositivity, a marker of high-risk sexual behaviour. We also conducted stratified analyses according to age. The level of statistical significance was p<0.05.

Paper IV

Immunogenicity analyses were based on the according-to-protocol (ATP) cohort. The analyses included all women who met the eligibility criteria, received the three vaccination shots and complied with protocol procedures. Seropositivity rates for HPV16 and HPV18 were calculated. *P-values* were calculated with Mann-Whitney test to compare mean absorbance of anti-HPV16 and HPV18 antibodies between non-smokers and smokers at each timepoint in the vaccine and control groups. Mean absorbance level for each antigen was reported with standard deviation (±Sd). Statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, USA).

5 RESULTS

5.1 Tobacco smoking as risk factor for invasive cervical cancer (Paper I)

Effect of increasing tobacco smoke exposure on cervical cancer risk

We found that the relative risk of CC increased with increasing serum cotinine levels. In both Study I cohort and Study II cohort serum cotinine level ≥100ng/ml, which indicated heavy smoking among females was associated with an increased risk of invasive CC (OR = 1.7, 95% CI = 1.2–2.5; OR = 2.5, 95% CI = 2.0–3.0 respectively). In the Study II cohort the HPV16 and 18 adjusted OR of SCC was markedly stronger in women with cotinine level ≥100ng/ml: 3.2 (95% CI = 2.6–4.0) compared to women with cotinine level 20-<100ng/ml: 2.2 (95% CI = 1.7–2.8) with only negligible overlap of the 95% CIs. Further adjustment for antibodies to HPV16/18, HSV-2 and *C. trachomatis* had no material effects on the point estimates. In both study cohorts, no serum cotinine levels indicative of smoking were associated with an excess risk of cervical adenocarcinoma.

Effect of increasing tobacco smoke exposure on cervical cancer risk among HPV16/18-seropositive and seronegative women

The large sample size of the Study II cohort allowed stratified analysis by HPV status among cases and controls. In HPV16/18-seropositives, significantly increased relative risk of invasive CC and of SCC were noted in women with cotinine level ≥100ng/ml (OR = 2.1, 95% CI = 1.4–3.2, OR = 2.7, 95% CI = 1.7–4.3 respectively). Adjustment for antibodies to HSV-2 and *C. trachomatis* had no effect on the point estimates. Among HPV16/18-seropositive women, cotinine level 20-<100 ng/ml was not associated with the risk of invasive CC (OR 1.1, 95% CI = 0.7–1.8). In HPV16/18-seronegatives, an increased relative risk of invasive CC and SCC was observed both in women with cotinine level 20-<100 ng/ml and ≥100 ng/ml (Table 5.1).

Table 5.1 Odds ratios (OR) for risk of cervical cancer in human papillomavirus (HPV) type 16 and/or 18 seropositive and seronegative non-smokers and smokers by increasing tobacco smoke exposure (defined as blood cotinine level), crude and adjusted for antibodies to herpes simplex virus type 2 (HSV-2) and *Chlamydia trachomatis* (Study II)

Cotinine (ng/ml)	Invasive cervical cancer				Squamous cell carcinoma			
	Crude OR	95% CI	OR adj*	95% CI	Crude OR	95% CI	OR adj*	95% CI
	HPV type 16 and/or 18 seropositives							
	253 cases		247 cases		202 cases		197 cases	
	597 controls		589 controls		460 controls		452 controls	
<20	1		1		1		1	
20-<100	1.1	(0.7, 1.8)	1.1	(0.7, 1.8)	1.2	(0.7, 2.0)	1.1	(0.6, 2.0)
100+	2.1	(1.4, 3.2)	2.1	(1.4, 3.2)	2.7	(1.7, 4.3)	2.7	(1.7, 4.4)
P value†	<0.001		<0.001		<0.001		<0.001	
	HPV type 16 and/or 18 seronegatives							
	335 cases		330 cases		243 cases		240 cases	
	2261 controls		2234 controls		1706 controls		1686 controls	
<20	1		1		1		1	
20-<100	2.0	(1.6, 2.6)	1.8	(1.4, 2.3)	2.7	(2.0, 3.6)	2.2	(1.7, 3.1)
100+	2.6	(2.0, 3.4)	2.3	(1.8, 3.0)	3.4	(2.5, 4.5)	2.9	(2.2, 4.0)
P value†	0.04		0.05		0.06		0.06	

* OR adjusted for herpes simplex virus type 2 (HSV-2) and *C. trachomatis*.
† Test for dose-response relationship (see statistical methods).

5.2 Tobacco smoking and chewing as risk factors for cervical SIL (Paper II)

The proportion of women exposed to tobacco was almost ten times higher in Finland (36.8%) than in Côte d'Ivoire (3.7%). In Côte d'Ivoire, tobacco chewing was more common than tobacco smoking (2.6% versus 1.4%), and concerned mostly women ≥30 years of age (4.8%).

Among tobacco chewers (Côte d'Ivoire), the risk of HSIL was five times higher in both young (OR = 5.5, 95% CI = 1.2–26) and older (OR = 5.5, 95% CI = 2.1–14) women compared to non-chewers (Table 5.2). Overall, the age-adjusted relative risk of cervical HSIL was also significantly increased among tobacco chewers (OR = 5.6, 95% CI = 2.5–12). In Finland, tobacco smokers (Finland) ≥30 years of age tended to have an increased risk of LSIL (OR = 2.5, 95% CI = 0.9–6.8) and HSIL (OR = 2.2, 95% CI = 0.5–8.7) (Table 5.2).

Table 5.2 Age-stratified tobacco smoking/chewing-associated OR with 95% CI of low-grade lesions and high-grade lesions in women from Côte d'Ivoire and Finland

Variables	Low-grade lesions		High-grade lesions	
	OR	(95% CI)	OR	(95% CI)
Côte d'Ivoire	*n = 165*		*n = 71*	
Age < 30 years				
Non-chewers	1.0		1.0	
Chewers	n.a		5.5	(1.2–26)
Age ≥ 30 years				
Non-chewers	1.0		1.0	
Chewers	2.0	(0.7–5.8)	5.5	(2.1–14)
Finland	*n = 60*		*n = 28*	
Age < 30 years				
Non-smokers	1.0		1.0	
Smokers	1.2	(0.6–2.5)	1.1	(0.4–3.1)
Age ≥ 30 years				
Non-smokers	1.0		1.0	
Smokers	2.5	(0.9–6.8)	2.2	(0.5–8.7)

Further, analyses were restricted to HPV DNA-positive women to assess independent tobacco smoking- and chewing-associated risk of cervical LSIL and HSIL. Despite the small numbers of women in both settings, we found an increased, albeit not significant, relative risk of both LSIL and HSIL, in HPV DNA-positive women ≥ 30 years of age and actively exposed to tobacco through smoking (OR = 2.2, 95% CI = 0.5–9.0 and OR = 1.8, 95% CI = 0.3–9.6 respectively) or chewing (OR = 2.3, 95% CI = 0.2–27 and OR = 7.3, 95% CI = 0.8–68, respectively).

5.3 Tobacco smoking and chewing as risk factors for multiple HPV infections (Paper II)

Being infected with two or more HPV types (multiple HPV infections) was common in HPV16 and/or 18-seropositive women (60.4% in Finland and 47.2% in Côte d'Ivoire). Antibodies to three HPV types or more were detected in 20 of 57 (35.1%) Ivorian and 50 of 129 (38.7%) Finnish women. The logistic regression analyses, however, did not show any increased risk of multiple infections among tobacco smokers/ chewers compared to non-smokers/chewers.

5.4   Effect of tobacco smoking on the immune response following genital HPV16/18 infection (Paper III)

Effect of smoking on the development of HPV16/18 antibody response

We assessed the effect of smoking on the development of HPV16/18 antibodies in initially HPV16/18 seronegative women as odds ratio. In women under 30 years of age, only three out of 29 (10%) smokers turned positive for at least one HPV16/18 antibody isotype (IgG/IgA) compared to 13 of 41 (32%) non-smokers (OR, 0.2; 95% CI 0.0–0.9) (Table 6). Smoking-associated relative risk of HPV16/18 seroconversion was not significantly decreased in the older age group (Table 5.3). There was no difference between HPV DNA positive or negative women with regard to the development of HPV antibodies.

TABLE 5.3 Smoking-associated relative risk (OR and 95% CI) of developing HPV16/18 antibodies in initially seronegative females with cytological atypia consistent with HPV infection

HPV16/18 seronegative at baseline	Change in serostatus n/N[d] (%)	OR (95%CI)	OR[a] (95%CI)
Age < 30 years			
IgA only			
Non-smokers[b]	7/47 (15)	1	1
Smokers[c]	1/32 (3)	0.1 (0.0–1.5)	0.2 (0.0–1.7)
IgG only			
Non-smokers	3/37 (8)	1	1
Smokers	2/20 (10)	1.2 (0.1–8.2)	1.1 (0.1–7.6)
IgA and IgG			
Non-smokers	13/41 (32)	1	1
Smokers	3/29 (10)	0.2 (0.0–0.9)*	0.2 (0.0–0.9)*
Age ≥ 30 years			
IgA only			
Non-smokers[b]	2/16 (12)	1	1
Smokers[c]	3/8 (37)	4.2 (0.5–32)	4.8 (0.3–59)
IgG only			
Non-smokers	2/26 (8)	1	1
Smokers	1/20 (5)	0.6 (0.1–7.5)	0.7 (0.1–8.4)
IgA and IgG			
Non-smokers	2/15 (13)	1	1
Smokers	2/7 (28)	2.6 (0.3–23)	3.2 (0.2–45)

[a] OR adjusted for Chlamydia trachomatis.
[b] Cotinine <20ng/mL; reference group.
[c] Cotinine ≥20ng/mL.
[d] n = number of females who changed their serostatus over time.
N = total number of non smoking and smoking females in each strata.

Effect of smoking on the stability of HPV16/18 antibody positivity

We assessed the smoking-associated relative risk of maintaining HPV16/18 antibody positivity in initially HPV16/18 seropositive women. We found a significantly decreased risk of constant IgG antibody positivity (OR, 0.1; 95% CI 0.0–0.8), based on one seroconversion case, among smokers. In women under 30 years of age, 13 of 21 (62%) smokers remained IgA or/and IgG positive until the end of follow-up compared to 20 out of 26 (77%) non smokers (Table 5.4). Smoking did not materially affect the risk of IgG antibody waning in women over 30 years of age (Table 5.4).

TABLE 5.4. Smoking-associated relative risk (OR and 95%CI) of persistence of HPV16/18 antibodies in initially seropositive females with cytological atypia consistent with HPV infection

HPV16/18 seropositive at baseline	No change in serostatus n/N[d] (%)	OR (95%CI)	OR[a] (95%CI)
Age < 30 years			
IgA only			
Non-smokers[b]	4/10 (40)	1	1
Smokers[c]	7/11 (64)	2.6 (0.4–15)	2.8 (0.5–17)
IgG only			
Non-smokers	8/12 (67)	1	1
Smokers	1/8 (12)	0.1 (0.0–0.8)*	0.1 (0.0–0.8)*
IgA and IgG			
Non-smokers	20/26 (77)	1	1
Smokers	13/21 (62)	0.4 (0.1–1.7)	0.4 (0.1–1.7)
Age ≥ 30 years			
IgA only			
Non-smokers[b]	7/18 (39)	1	1
Smokers[c]	2/6 (33)	0.2 (0.0–6.9)	0.2 (0.0–6.8)
IgG only			
Non-smokers	10/14 (71)	1	1
Smokers	3/4 (75)	1.2 (0.1–15)	1.2 (0.1–15)
IgA and IgG			
Non-smokers	22/36 (61)	1	1
Smokers	9/11 (82)	2.8 (0.5–15)	2.8 (0.5–15)

[a] OR adjusted for Chlamydia trachomatis.
[b] Cotinine <20ng/mL; reference group.
[c] Cotinine ≥20ng/mL.
[d] n = number of females who changed their serostatus over time.
N = total number of non smoking and smoking females in each strata.

5.5 Effect of smoking on the immune response following prophylactic HPV16/18 AS04-adjuvanted VLP vaccination

A total of 216 young women from the Finnish arm were included in this pilot study. Out of 103 participants from the HPV16/18 group, 46.6% (48) were smokers compared to 56.6% (64) smokers in the control group.

We evaluated differences in mean absorbance of anti-HPV16 and HPV18 antibodies levels from baseline to month 7 post-vaccination. The results showed that the mean absorbance of anti-HPV16 antibody levels was 1.97 (±0.78) among non-smokers compared to 1.88 (±0.73) among smokers. The mean absorbance of anti-HPV18 antibody levels was 1.44 (±0.85) among nonsmokers and 1.36 (±0.76) among smokers. The observed differences between the two groups were not statistically significant (Table 5.5).

TABLE 5.5 Comparison of mean absorbance level (with standard deviation (Sd)) of anti-HPV16 and HPV18 antibodies between. Non-smokers and smokers by vaccination group

	Vaccine (n = 103)			Placebo (n = 113)		
	Non-smokers (n = 55)* mean (±Sd)	Smokers (n = 48)* mean (±Sd)	*p-value*†	Non-smokers (n = 49)* mean (±Sd)	Smokers (n = 64)* mean (±Sd)	*p-value*†
HPV16 Baseline Month7	0.12 (± 0.08) 1.97 (± 0.78)	0.29 (± 0.52) 1.88 (± 0.73)	0.2 0.4	0.21 (± 0.43) 0.01 (± 0.04)	0.18 (± 0.29) 0.01 (± 0.02)	0.9 0.7
HPV18 Baseline Month7	0.07 (± 0.06) 1.44 (± 0.85)	0.18 (± 0.08) 1.36 (± 0.76)	0.1 0.6	0.10 (± 0.08) 0.01 (± 0.03)	0.10 (± 0.14) 0.01 (± 0.03)	0.8 0.7

* Nonsmokers (cotinine < 20ng/ml) and smokers (cotinine ≥ 20 ng/ml).
† *p-value* derived from Mann-Whitney test to evaluate mean absorbance difference between non-smokers and smokers.

6 DISCUSSION

6.1 Tobacco exposure of fertile-aged women

There are an estimated 1.3 billion adult smokers (over 15 years old) among the world's six billion people. If the prevalence of tobacco use remains constant, the number of smokers will rise to 1.7 billion between 2020 and 2025 (Guindon and Boisclair 2003). Four-fifths of current smokers live in low-income or middle-income countries.

There are important gender-specific differences in tobacco use, with global prevalence among males (48%) about four times higher than among females (10%) (Guindon and Boisclair 2003). There may, however, be considerable female smoking that is underreported, or unreported, because of gender norms that stigmatize women who smoked. Male-female differences in use are the highest in the Western Pacific Region and the lowest in the Americas and Europe, where about one quarter of women smoke (Carrao et al. 2000).

Typically, the smoking epidemic starts in most populations among men and higher-income groups, and later affects women and low-income groups. However, global male rates have peaked and have stabilized or are in slow decline, while the prevalence of tobacco use among women is increasing (Mackay 2001, Molarius et al. 2001). In fact, the historical gender differences in uptake and prevalence are diminishing because of the increased prevalence of smoking among adolescent women. Recent findings of the GYTS, the largest global survey of adolescents (aged 13 to 15) and tobacco use, show that almost as many adolescent girls and adolescent boys are smoking in many parts of the world (GYTS Collaborating Group 2003). This is an indication of the increasing global smoking epidemic among women that will not peak until well into the 21st century. The prediction is that by 2025, 20% of the female population will be smokers, up from 12% in 2005.

Tobacco exposure is a potential environmental cofactor of CC. Therefore, the high frequency of tobacco smoking and chewing among women emphasizes the importance of assessing its impact on the development and prevention of cervical neoplasia.

6.2 Comparison of the study with findings from other studies

6.2.1 Tobacco smoking is an independent risk factor for cervical cancer

Smoking was associated with an increased risk of ICC/SCC after both adjustment and stratification for antibodies to oncogenic HPVs. While the former could be confounded by misclassification bias in the serological diagnosis of hrHPV infection, the latter provided estimates unconfounded by persistent hrHPV infection as determined by serology with up tp 75% sensitivity in cases and controls (Kjellberg et al. 1999). The Nordic joint study (Pukkala et al. 2007) is by far the largest nested case-control study on risk factors of cervical cancer. Study I and Study II showed significant increased risk of ICC/SCC among women with cotinine level >100mg/ml. We found that the point estimate was lower in Study I relative to Study II. Women were from the same population and the same design was applied in both studies. Chance could be one explanation of this observed difference. Another explanation could be the age difference since the Study II material included older women compared with the Study I material. The carcinogenic effect of smoking is likely to increase with inceased age at diagnosis (This study, Plummer et al. 2003) and is usually observed after a long period of exposure to tobacco smoke (Gunnell et al. 2006). The Study II material allowed stratified analyses to assess the independent role of smoking. We found a significantly increased two-fold excess risk of SCC, free of the non-differential residual confounding bias, among HPV16/18-seropositive women.

HPV VLP serology has been proven to be a specific albeit not sensitive marker of current and past exposure to HPV (Olsen et al. 1997). It permitted the delineation of HPV16/18-infected (seropositive) controls that could be compared with HPV16/18-infected (seropositive) cases. Our results are consistent with the few prospective studies that have controlled for HPV infection using high quality HPV assays (Plummer et al. 2003). In the UK, Deacon et al. analySed risk factors for progression to CIN 3 among HPV-positive women in a nested case-control study and reported a high risk of 2.2 (95% CI = 1.4–3.4) for ever smoking with a strong evidence of a dose-response effect.

Several case-control studies (Bosch et al. 1992, Eluf-Neto et al. 1994, Chaouki et al. 1998, Santos et al. 2001, Hildesheim et al. 2001, Olsen et al. 1998, Rolon et al. 2000), including the IARC multicentre pooled analysis of 10 studies of ICC (Plummer et al. 2003), examined tobacco smoking as a co-factor to HPV infection by restricting the analysis to HPV DNA positive study participants. The results from these analyses showed no significant alteration in risk of CIN or CIS whether or not the study participants were HPV-DNA-positive. Most of these studies, however, tested for HPV-DNA at only one timepoint, which favoured the detection

of amplified HPV DNA in the cases. Furthermore, controls testing HPV-positive just once most likely have a transient infection. This could bias the measure for lifetime exposure and impact of HPV infection. Such a problem does not exist for HPV-seropositives since the possibility that the HPV antibodies wane over time is highly unlikely (af Geijersstam et al. 1998).

The presence of tobacco carcinogens in cervical mucus has been described as a possible biological explanation for the epidemiological association (Simons et al. 1995). The mechanism could involve soluble carcinogens that may have a direct transforming effect on squamous cervical epithelium. Some other epithelial cancers, those of the nasal cavity and the oesophagus, show similar differences between SCC and adenocarcinoma with regard to smoking and HPV (IARC 2004, Björge et al. 1997, Mork et al. 2001). Cigarette smoking may also exacerbate the carcinogenic potential of HPV specifically via inhibition of IFN-gamma and/or tumour necrosis factor alpha, leading to a significant inhibition of the apoptosis, which may promote tumor growth (Suk et al. 2001, Hussain et al. 2008). Experimental studies show that cigarette smoking decreases the secretion of proinflammatory cytokines (IL1, IL6) and suppresses IL2 and IFNγ production (Ouyang et al. 2000, Arcavi 2004), suggesting the possibility that cigarette smoking interacts to increase cervical carcinogenesis development.

6.2.2 Tobacco use (smoking/chewing) and the risk of cervical SIL

The route of exposure to tobacco was different between Côte d'Ivoire and Finland probably due to socio-economic and cultural differences. In addition, screening for cervical cancer and laboratory analyses for HPV detection were performed in two different settings and at different times. The differences observed in the association of tobacco exposure and SIL by the route of tobacco exposure may, however, be valuable for future studies. Comparison of the separated results was feasible since the method used for the PAP smear test was comparable, and assessment and grading of cervical SIL was based on a comparable method. The lesions were classified into LSIL and HSIL according to the Bethesda System.

In the Côte d'Ivoire material, we identified a lack of statistically significant association between tobacco chewing and the risk of cervical LSIL in Ivorian women ≥30 years of age. Despite the low prevalence of tobacco use, our results showed that women who chewed tobacco had a significantly increased risk of cervical HSIL. To date, only one study has reported an association between tobacco chewing and increased risk of invasive CC in Southern India (Rajmakar et al. 2006). Smokeless tobacco products contain and deliver high quantities of nicotine comparable to those typically absorbed from cigarette smoking. Moreover, these products are carcinogenic to humans and may result in local and systemic health hazards depending of the way of administration and the content of various toxic

products, including nicotine and tobacco-specific nitrosamines (Hoffman and Djordjevic 1997, Cogliano et al. 2004).

There was an increased risk of LSIL and HSIL among Finnish women ≥ 30 years of age who smoked tobacco. This is in line with recent studies showing that tobacco smoking increases the risk of cervical SIL and CC (Kjellberg et al. 2000, McIntyre-Seltman et al. 2005, Tolstrup et al. 2006). The point estimates, however, did not reach statistical significance possibly due to the small number of cases.

The general degree of concordance between PCR and ISH is between 70 and 90%, the main difference yielding in the sensitivity of the methods particularly among patients with high-grade lesions (Takuma et al. 2005, Dabie et al. 2004, Biedremman et al. 2004). The failure of HPV DNA hybridization studies to find all cervical HPV infections has been known for a long time (Leminen et al. 1992). Possible recall bias may have led to under-reporting of smoking habits but it is unlikely to have affected the results as the accuracy of self-reported tobacco smoking habit is generally high in Finland (Vartiainen et al. 2002).

We further restricted the analyses to HPV DNA-positive women to assess the independent role of tobacco smoking and chewing in cervical SIL. Although Ivorian and Finnish women ≥30 years of age tended to have an increased risk of LSIL and HSIL, we could not draw any conclusion due to the small number of women. Syrjänen et al. did not report an increased risk of high-grade CIN among smokers (Syrjänen et al. 2007). It is possible that being exposed to tobacco through smoking or chewing is not an independent risk factor of cervical SIL as the increased risk could be mediated by acquisition of hrHPV infection (Syrjänen et al. 2007). When restricting the analyses to women who were HPV DNA-positive, it is possible that we restricted attention to women whose immune response to HPV was partially inadequate and, in whom, therefore, smoking and chewing status could not have had an additional effect (Rousseau et al. 2003).

6.2.3 Tobacco use (smoking/chewing) and the risk of multiple HPV infections

There was no association between tobacco use (smoking and chewing) and the risk of multiple HPV infections. Kataja et al. previously reported that current smoking was a strong risk factor for HPV infection among Finnish women, although the risk did not increase in parallel with the number of cigarettes and years smoked (Kataja et al. 1993). More HPV types were detected in Côte d'Ivoire than in the Finnish study, but similar negative findings were reported among tobacco chewers or smokers. In Canada also, Rousseau et al. did not find an association between tobacco smoking and multiple HPV infections among women (Rousseau et al. 2003). No data have previously been published on the association between smoking/chewing and the risk of multiple HPV infections among women from less industrialized countries.

6.2.4 Tobacco smoking impairs the immune response following genital HPV16/18 infections

To the best of our knowledge, this is the first follow-up study to relate smoking status based on cotinine measurements to the likelihood of developing or maintaining HPV16/18 antibody positivity. Young women who smoked were less likely to either seroconvert or maintain detectable HPV16/18 antibodies over time relative to non-smokers.

Smoking impairs protection against and/or immunosurveillance of HPV infections in the uterine cervix (Syrjänen et al. 2007, Wiley et al. 2006). In a cross-sectional study, HPV16 DNA-positive women aged 16 to 23 years who smoked were less likely to test positive for HPV16 IgG antibodies than non-smokers (OR, 0.5; 95% CI 0.3–0.9) (Wiley et al. 2006). The authors suggest that there was a dose-response relationship between smoking and anti-HPV16 antibody positivity. In our study the observed effect of smoking was possibly also due to the high tobacco exposure in young women, who tended to be heavy smokers. The follow-up time was not the same for all the women (range: 6–114 months) and this difference may have an effect on the point estimates. This is however unlikely, as there was no difference in the length of follow-up time between HPV-seronegative and HPV-seropositive women at last sampling (Chi squarre (χ^2) = 14.46, df = 12, p = 0.2). In addition, we did not observe a difference in the length of follow-up time between non-smokers and smokers (χ^2 = 7.11, df = 12, p = 0.8).

Seroconversions against the HPV16 and 18 capsids are seen within up to a year following primary HPV16 and 18 infections (Wang et al. 2000, af Geijeirsstam et al. 1998), and IgA antibodies appear in a maximum of 20 months (Onda et al. 2003). Our findings showed that tobacco exposure may significantly delay the development of HPV16/18 IgA or/and IgG antibodies in young women followed-up for up to 5 years. It is commonly known that smokers have 10 to 20% lower serum antibody (IgA, IgG and IgM) levels than non-smokers. The immunoglobulin levels are higher among past than current smokers, and increase with duration of smoking cessation (Anderson et al. 1982). HPV-specific antibody response is often delayed but our cohort study, with a long follow-up time, allowed us to make the distinction between females who, eventually, had responded and those who had failed to respond over time. Determination of HPV16/18 IgA and IgG antibodies was by standard ELISA (Pagliusi et al. 2007). Serum antibodies are not affected by differences in blood withdrawal or specimen handling (Lehtinen et al. 2006). Following natural HPV infection, IgG antibodies are detectable in up to 75% of women (Kjellberg et al. 1999). HPV16/18 IgG seropositive women <30 years who smoked, had a significantly decreased likelihood of being constantly IgG antibody positive suggesting that smoking may promote waning of IgG antibodies over time.

Interpreting the results was not straightforward due to the small sample size. Our old HPV DNA typing data probably distinguished HPV positive cases with high viral load only. This did not seem to have an effect as the results in DNA positive

and DNA negative women were comparable. However, having few strata with small numbers of individuals cannot exclude the possibility of change observations.

How tobacco exposure specifically influences HPV antibody response is not known. HPV-infected cells may also be more susceptible to DNA damage from specific tobacco carcinogens (Simons et al. 1995). In addition, the fact that some cigarette constituents have the ability to manipulate cytokine expression in a manner similar to hrHPV gene expression suggests that smoking/chewing may enhance the ability of HPV16/18 to escape from immune surveillance (Poppe et al. 1995, Arcavi et al. 2004, Hussain et al. 2008). Smoking has been associated with persistent HPV infections (Szarewski et al. 2001) and decreased clearance of CIN lesions (Szarewski et al. 1996). Reduction in lesion size was highly correlated both with the reduction in reported amount of smoking and with the final-visit cotinine measurements (Szarewski et al. 1996).

6.2.5　Tobacco smoking may not impair the immune response following bivalent HPV16/18 vaccination

This pilot study is the first to evaluate the impact of tobacco smoking, assessed via serum cotinine level, on the immunogenicity of bivalent HPV16/18 AS04-adjuvanted vaccine. Young women who smoked tended to have comparable absorbance levels of anti-HPV16 and HPV18 antibodies to non-smokers at 7 months-post-vaccination.

In contrast to the often inadequate immune response following natural infection, a prophylactic CC vaccine should induce long-term immune response with high and sustained local and systemic antibody levels (Stanley et al. 2006). The addition of an adjuvant to a vaccine significantly increases the humoral response to the vaccine antigens (Giannini et al. 2006). Geometric titers (GMTs) for vaccine induced antibodies against HPV16 or HPV18 infections were substantially higher (≥11-fold) than those seen in natural HPV16 or HPV18 infections at all timepoints for 5.5 years (Harper et al. 2006). High seroconversion rates and strong vaccine-induced antibody response were also evident in the interim (14.8-month) results of the PATRICIA study, a large phase III trial of the AS04-adjuvanted HPV16/18 vaccine (Paavonen et al. 2007).

Our study demonstrated no statistically significant differences in vaccine-induced humoral immune response to either HPV16 or HPV18 L1 VLP among young women who smoked compared to non-smokers after a 7-month follow-up period. In a recent study investigating the effect of self-report smoking on HPV16/18 L1 VLP vaccine among small numbers of young women with preexisting HPV infections, the authors found no vaccine efficacy for viral clearance in either smokers (VE: -51.9, 95% CI: -136.7 to 2.5) or non-smokers (VE: 6.2, 95% CI: -17.1 to 24.8) after 12 months of follow-up (Hildelsheim et al. 2007). A similar observation has also been described for the quadrivalent HPV6/11/16/18 vaccine (Giuliano et al. 2007).

Smoking is a risk factor for oncogenic HPV infections (Syrjanen et al. 2007) and cervical neoplasia (Plummer et al. 2003). Tobacco smoking prevalence has increased considerably among women during the last decade (Mackay 2001). Long-term immunogenicity and efficacy of HPV vaccine in preventing HPV infection and cervical neoplasia is unclear (Keam and Harper 2008). Although this pilot study was limited by the short follow-up period, the results showed that both the anti-HPV16 and anti HPV18 absorbance levels in HPV16/18-vaccinated smokers and non-smokers were comparable at month 7 post-vaccination. Comparison between the type-specific ELISAs determined antibody levels is, unfortunately, not possible. These observations, however, warrant further investigation on a larger number of vaccinated individuals.

6.3   Strengths and limitations of the study

Study design and bias

Case-control studies are susceptible to various forms of bias compared to cohort studies, the usefulness of the former in the process of causal inference is especially diminished. Our nested case-control design (Paper I) measured data on exposure and confounders before the diagnosis of the disease, thus reducing potential recall bias (pregnant women from FMC were a part of the material), the temporary ambiguity usually inherent in case-control studies. Potential recall bias may be a limitation in the second study (Paper II) as under-reporting of tobacco smoking habit among Finnish women may have distorted the true association between exposure and cervical HSIL. This is, however, unlikely because we found a good correlation between serum cotinine levels and the questionnaire data among Finnish women (data not shown).

Cases and controls were drawn from the same cohort, decreasing the likelihood of selection bias. In Papers II and III, we performed cross-sectional studies. Despite the fact that this prevents us from concluding the directionality of the observed associations, these cross-sectional analyses allowed hypothesis generation for future research.

Paper IV described the results from a random subset of a randomized prospective clinical intervention study (Paavonen et al. 2007). Computer-generated randomization was performed to avoid selection bias. Vaccine and Placebo groups were equally treated following a standardized written protocol in order to minimize bias in study management. In addition, the doubled-blinded design made it possible to avoid observer (ascertainment) bias.

Misclassification of the outcome variables (Papers I and II) was unlikely as the diagnoses for cervical SIL, SCC or carcinoma in-situ (CIS) were reported by experts in cytopathology and mostly based on results from histology. Samples from cases (Paper I) and their controls were batched together during the laboratory analyses

to avoid assay-drift bias. Moreover, great care was taken to avoid differential measurement errors in the determination of HPV antibodies positivity or in cotinine detection by blinding the laboratory personnel (Papers I, II, III and IV).

Confounding

We identified some potentiel confounders on the basis of earlier studies and biology. In the statistical analyses, we did not have questionnaire data on sexual habits, which may have resulted in slight over-estimation of the smoking-associated relative risks. This is however, unlikely as the point estimates were adjusted for antibodies to *C. trachomatis*, a sensitive surrogate of risk-taking sexual behaviour. Seropositivity to *C. trachomatis* shows a strong correlation with life-time number of sexual partners (Dillner et al. 1996).

Validity of the method used for tobacco exposure assessment

Complete questionnaire data on tobacco exposure history would have permitted us to assess the lifetime exposure to tobacco smoke, and to study the effects of smoking in relation to age at starting and stopping smoking. Self-report has proven to be a simple and powerful predictor of disease outcomes in epidemiological studies. Self-reporting, however, underestimates the true prevalence and is inaccurate as to the quantity of smoking exposure (Murray et al. 1993). When smoking is a daily habit, the act of smoking is unlikely to be closely self-monitored. Thus, in a questionnaire, the respondent may be imprecise about smoking behaviours, such as number of cigarettes smoked per day (Benowitz 1999). On the other hand, pregnant women (a considerable part of our study cohorts in Studies I and II materials) tend to belittle their smoking habit because of health conscience (Britton et al. 2004, Burstyn et al. 2009).

Biochemical assessment of tobacco exposure integrates different aspects of the true exposure, including tobacco composition, uptake, distribution and individual differences in metabolism. Cotinine is a good marker of nicotine, which is the important carcinogen in tobacco smoke. Although serum cotinine measures recent exposure to tobacco smoke near the time of specimen collection, it has a high sensitivity and specificity for tobacco exposure and the measurement error is negligible compared to questionnaires (Murray et al. 1993, Boffeta et al. 2006). Biochemistry of identifying tobacco exposure via serum cotinine favours the validity of our observations. This method addresses the issue of non-differential recall bias. In addition, it is the inhaled dose of tobacco smoke that is directly related to the development of tobacco-related diseases (Pérez-Stable et al. 1995). Cotinine levels remain stable in frozen samples (Boffeta et al. 2006) enabling the identification of dose-response effects.

Validity of the results

The validity of the study refers to the reliability of the results. From the first to the fourth study, great care was taken to ensure that the results do not reflect the presence of bias or confounding. In biobank-based research, the process from the decision to take a sample from an individual to the moment the sample is safely placed in the biobank consists of several phases including collection of samples, handling and storage of samples. Failure in one of these standardized procedures may have significant consequences as the quality of the material stored in the biobank as well as conclusions and recommendations based on the analysis of such material may be severely affected. Established quality control of serums banks and cancer registries (Paper I) increases the validity of the study (Pukkala et al. 2007, Sjöholm et al. 2007).

Sample size and generalisability of the results

Our Nordic joint study (Paper I) is by far the largest nested case-control study on risk factors for CC conducted to date. Updating the study material (Study I) eventually result in a sample size large enough to provide confidence in the estimates and therefore, ensure our ability to reveal associations. The population representativeness of the serum bank cohorts was recently assessed by Pukkala and colleagues (Pukkala et al. 2007) who found no significant difference in cancer incidence in the biobank cohorts involved in the present study (the Nordic maternity cohorts and the Janus biobank) as compared with respective national rates. Thus, our results can be generalized to all Nordic women, and there was no evidence that the odds ratios among serum banks were heterogeneous.

In Papers II and III, the cohort participants were recruited among women attending routine Papanicolaou (PAP) smear. The screening activities are integrated in the healthcare system in Finland. In the organised programme, the present coverage of invitations is 90% of the target age; the participation rate is higher than 70% (Anttila and Nieminen 2000). In our study, all women whose PAP smear showed unequivocal changes of HPV infection attended the gynecological outpatient clinic of the Kuopio University Hospital for follow-up visits at 6-month intervals. There was no loss to follow-up in the Finnish study. In Cote d'Ivoire (Paper II) cases were drawn from the three outpatient gynecology clinics in Abidjan and may not accurately reflect the target population.

Assuming interval validity (Papers II to IV) and despite small sample size (Papers II and III), our results should also be representative of the Finnish (Papers II to IV) and Ivorian (Paper II) targeted populations. The study groups had similar physiological, behavioral, racial and cultural characteristiques than their respective general population. Caution must be exercised, however, in extending these results to other countries citizens as numerous other differences exist between nations in relation to genetics and lifestyle factors.

7   CONCLUSIONS

Tobacco smoking was associated with an increased risk of CC/SCC after adjustment to oncogenic HPVs. Moreover, a statistically significant 2-fold excess risk of SCC was found among HPV16/18-seropositive women who smoked. The risk of CC associated to smoking was similar in seropositive and seronegative women.

Tobacco smokers tended to have an increased risk of HSIL in women ≥30 years of age. In less industrialized countries, which bear the heaviest burden of CC, exposure to tobacco is more common through chewing than smoking among women. Relative to non-chewers, women who chewed tobacco had a significantly 5-fold increased risk of cervical HSIL. Although being infected with two or more HPV types (multiple infections) was common in HPV16 and/or HPV18-infected women, we did not report an increased risk of multiple HPV infections among tobacco smokers/chewers compared to non-smokers/non-chewers.

We showed that following natural HPV infection, young women who smoked were less likely to either seroconvert or maintain detectable HPV16/18 antibodies over time than non-smokers.

We found that following AS04-adjuvanted HPV16/18 L1 VLP vaccination, young women who smoked or did not smoke had comparable short-term HPV16/18 serum antibody response at 7 months post vaccination.

We conclude that tobacco smoking is an independent risk factor for CC in women infected with oncogenic HPVs. In addition, the results suggest that smoking may induce impaired antibody response to oncogenic HPVs but not immediately after prophylactic HPV vaccination in young women. The evidence discussed in this dissertation supports comprehensive primary prevention initiatives against causes of CC, with a call for strengthening efforts to prevent tobacco smoking and chewing exposures among women.

8 ACKNOWLEDGEMENTS

First of all, I would like to acknowledge my supervisor, Research Prof. Matti Lehtinen, for his excellent guidance and advice throughout my PhD studies. His inspiring research ideas, optimistic attitude and devotion for research have been a great source of motivation. I have always received from him immediate comments of the manuscripts and prompt feedback to all my e-mail questions. He has provided me with an excellent example to follow in my career.

I express my sincere thanks to the reviewers, Research Prof. Jarmo Virtamo and Adjunct Professor Pekka Nieminen, for their careful review and comments on this manuscript. I am indebted to Prof. François Dabis, Université Victor Segalen Bordeaux 2, for giving me the data of the study conducted in Côte d'Ivoire and for his encouragement. I am deeply grateful to Dr. Heljä-Marja Surcel for sharing her expertise in immunology, coordinating the laboratory analyses and giving valuable comments on the manuscripts. With her endless support and excellent advice on all aspects of life, she has contributed to my social well-being while in Finland. I also express my sincere thanks to my other co-authors whose pleasant cooperation and encouragement made this work possible.

I wish to express my sincere thanks to all members of CCPRB network especially Prof. Joakim Dillner, Prof Göran Hollmans, Prof. Timo Hakulinen, Dr. Laufey Tryggvadóttir. My special thanks go to Mr. Tapio Luostarinen for his valuable contributions regarding statistical analyses and manuscript preparation. My special thanks go to Dr Anneli Pouta, head of the department, THL Oulu, for providing facilities. I am very grateful to Ms. Hanna Öhman, Ms. Marjo Kaasila, Ms. Taimi Aino and Mr. Matti Kesti for always being available for me. I deeply thank all the staff of the serological laboratory especially Ms. Pirjo Kontiotkari, Ms Aljona Amelia, Ms Mari Päätalo and Ms Annika Uimanen for their excellent technical support. It has been a great pleasure and honour working with you all. Thank you to Dr Tarja Kaijalainen for her constant encouragement. I wish to express my sincere thanks to Olavi and Raili Voittonen for their great hospitality and friendship and for showing us the beauty of Oulu.

My special thanks go to Prof. Pekka Rissanen, Director of TSPH, for giving me the opportunity to study here. I express my sincere thanks to all the lecturers of TSPH for teaching and sharing their valuable knowledge. I am very grateful to Ms. Tuula Joof, Ms. Anna-Mari Nykånen and Ms. Catarina Ståhle-Nieminen for their excellent assistance in all administrative issues related to my studies as well as my

References

af Geijersstam V, Kibur M, Wang Z, Koskela P, Pukkala E, Schiller J, Lehtinen M and Dillner J (1998): stability over time of serum antibody levels to human papillomavirus type 16. J Infect Dis 177:1710–1714.

Agarwal SS, Sehgal A, Sardana S, Kumar A and Luthra UK (1993): Role of male behavior in cervical carcinogenesis among women with one lifetime sexual partner. Cancer 72:1666–1669.

Agosti JM and Goldie SJ (2007): Introducing HPV vaccine in developing countries--key challenges and issues. N Engl J Med 356:1908–1910.

Al-Delaimy WK, Mahoney GN, Speizer FE and Willett WC (2002): Toenail nicotine levels as a biomarker of tobacco smoke exposure. Cancer Epidemiol Biomarkers Prev 11:1400–1404.

Al-Delaimy WK (2002): Hair as a biomarker for exposure to tobacco smoke. Tobacco Control 11:176–182.

Ali S, Astley SB, Sheldon TA, Peel KR and Wells M (1994): Detection and measurement of DNA adducts in the cervix of smokers and non-smokers. Int J Gynecol Cancer 4:188–193.

Anderson P, Perderson OF, Bach B and Bonde GJ (1882): Serum antibodies and immunoglobulins in smokers and nonsmokers. Cinical Experimental Immunol 47:467–473.

Andersson-Ellström A, Dillner J, Hagmar B, Schiller J, Sapp M, Forssman L and Milsom I (1996): Comparison of development of serum antibodies to HPV16 and HPV33 and acquisition of cervical HPV DNA among sexually experienced and virginal young girls. A longitudinal cohort study. Sex Transm Dis 23:234–238.

Antilla A and Nieminen P (2000). Cervical cancer screening programme in Finland. Eur J of Cancer 36: 2209–2214.

Anttila A, Pukkala E, Nieminen P and Hakama M (1998): Incidence of cervical cancer is clearly increasing in Finland. Duodecim114:1117–1124

Anttila T, Saikku P, Koskela P, Bloigu A, Dillner J, Ikäheimo I, Jellum E, Lehtinen M, Lenner P, Hakulinen T, Närvänen A, Pukkala E, Thoresen S, Youngman L and Paavonen J (2001): Serotypes of Chlamydia trachomatis and risk for development of cervical squamous cell carcinoma. JAMA 285:47–51.

Appleby P, Beral V, Berrington de GA, Colin D, Franceschi S, Goodhill A, Green J, Peto J, Plummer M and Sweetland S (2007): Cervical cancer and hormonal contraceptives: collaborative reanalysis of individual data for 16,573 women with cervical cancer and 35,509 women without cervical cancer from 24 epidemiological studies. Lancet 370:1609–1621.

Arbyn M, Anttila A, Jordan J, Ronco G, Schenck U, Segnan N, Wiener HG, Herbert A, Daniel J and von Karsa L (2008a): European guidelines for quality assurance in cervical cancer screening. Eds. European Commission, Luxembourg.

Arbyn M, Bergeron, Klinkhamer C, Martin-Hirsch P, Siebers P and Bulten J (2008b): Liquid compared with conventional cervical cytology: a systematic review and meta-analysis. Obstet Gynecol 111:167–177.

Arbyn M, Sankaranarayanan R, Muwonge R, Keita N, Dolo A, Mbalawa CG, Nouhou H, Sakande B, Wesley R, Somanathan T, Sharma A, Shastri S and Basu P (2008c): Pooled analysis of the accuracy of five cervical cancer screening tests assessed in eleven studies in Africa and India. Int J Cancer 123:153–160.

Ashrafi GH, Brown DR, Fife KH and Campo MS (2006): Down-regulation of MHC class I is a property common to papillomavirus E5 proteins. Virus Res 120:208–211.

Autier P, Coibion M, Huet F and Grivegnee AR (1996): Transformation zone location and intraepithelial neoplasia of the cervix uteri. Br J Cancer 74:488–490.

Auvinen E, Alonso A and Auvinen P (2004): Human papillomavirus type 16 E5 protein colocalizes with the antiapoptotic Bcl-2 protein. Arch Virol 149:1745–1759.

Bais AG, Beckmann I, Lindemans J, Ewing PC, Meijer CJ, Snijders PJ and Helmerhorst TJ (2005): A shift to a peripheral Th2-type cytokine pattern during the carcinogenesis of cervical cancer becomes manifest in CIN III lesions. J Clin Pathol 58:1096–1100.

Barnabas RV, Laukkanen P, Koskela P, Kontula O, Lehtinen M and Garnett GP (2006): Epidemiology of HPV 16 and cervical cancer in Finland and the potential impact of vaccination: mathematical modelling analyses. PLoS Med 3:e138.

Barnard P and McMillan NA (1999): The human papillomavirus E7 oncoprotein abrogates signaling mediated by interferon-alpha. Virology 259:305–313.

Bauman KE, Koch GG, Bryan ES, Haley NJ, Downtown MI and Orlandi MA (1989): On the measurement of tobacco use by adolescents. Validity of self-reports of smokeless tobacco use and validity of cotinine as an indicator of cigarette smoking. Am J Epidemiol 130:327–337.

Becona E and Vazquez FL (1998): Self-reported smoking and measurement of expired air carbon monoxide in a clinical treatment. Psychological Reports83:316–318.

Benowitz NL and Jacob P, 3rd (1994): Metabolism of nicotine to cotinine studied by a dual stable isotope method. Clinical Pharmacology and Therapeutics 56:483–493.

Benowitz NL (1996a): Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiologic Reviews 18:188–204.

Benowitz NL (1996b): Pharmacology of nicotine: Addiction and therapeutics. Annual Review of Pharmacology and Toxicology 36:597–613.

Beral V, Hannaford P and Kay C (1998): Oral contraceptive use and malignancies of the genital tract. Results from the Royal College of General Practitioners' Oral Contraceptive Study. Lancet 2:1331–1335.

Bhonsle RB, Murti PR, Gupta PC and Mehta FC (1976): Reverse dhumti smoking in Goa: An epidemiological study of 5,449 villagers for oral precancerous lesions. Indian Journal of Cancer 13:301–305.

Birkeland SA, Storm HH, Lamm LU, Barlow L, Blohmé I, Forsberg B, Eklund B, Fjeldborg O, Friedberg M, Frödin L, Glattre E, Halvorsen S, Holm NV, Jakobsen A, Jorgensen HE, Ladefoged J, Lindholm T, Lundgren G and Pukkala E (1995):. Cancer risk after renal transplantation in the Nordic countries, 1964-1986. Int J Cancer 60:183–189.

Biswas LN, Manna B, Maiti PK and Sengupta S (1997): Sexual risk factors for cervical cancer among rural Indian women: a case-control study. Int J Epidemiol 26:491–495.

Bjørge T, Hakulinen T, Engeland A, Jellum E, Koskela P, Lehtinen M, Luostarinen T, Paavonen J, Sapp M, Schiller J, Thoresen S, Wang Z, Youngman L and Dillner J (1997): A prospective, seroepidemiological study of the role of

human papillomavirus in oesophagal cancer in Norway. Cancer Res 57:3989–3992.

Block SL, Nolan T, Sattler C, Barr E, Giacoletti KE, Marchant CD, Castellsagué X, Rusche SA, Lukac S, Bryan JT, Cavanaugh PF Jr and Reisinger KS; Protocol 016 Study Group (2006): Comparison of the Immunogenicity and Reactogenicity of a Prophylactic Quadrivalent Human Papillomavirus (Types 6, 11, 16, and 18) L1 Virus-Like Particle Vaccine in Male and Female Adolescents and Young Adult Women. Pediatrics 118:2135–2145.

Boffetta P, Clark S, Shen M, Gislefoss R, Peto R and Andersen A (2006): Serum cotinine level as predictor of lung cancer risk. Cancer Epidemiol Biomarkers Prev 15:1184–1188.

Bontkes HJ, de Gruijl TD, Bijl A, Verheijen RH, Meijer CJ, Scheper RJ, Stern PL, Burns JE, Maitland NJ and Walboomers JM (1999): Human papillomavirus type 16 E2-specific T-helper lymphocyte responses in patients with cervical intraepithelial neoplasia. J Gen Virol 80:2453–2459.

Bontkes HJ, de Gruijl TD, van den Muysenberg AJ, Verheijen RH, Stukart MJ, Meijer CJ, Scheper RJ, Stacey SN, Duggan-Keen MF, Stern PL, Man S, Borysiewicz LK and Walboomers JM (2000): Human papillomavirus type 16 E6/E7-specific cytotoxic T lymphocytes in women with cervical neoplasia. Int J Cancer 1:92–98.

Bosch FX, Burchell AN, Schiffman M, Giuliano AR, de Sanjose S, Bruni L, Tortolero-Luna G, Kjaer SK and Muñoz N (2008): Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. Vaccine 26 Suppl 10:K1–16.

Bosch FX, Castellsague X, Munoz N, de SS, Ghaffari AM, Gonzalez LC, Gili M, Izarzugaza I, Viladiu P, Navarro C, Vergara A, Ascunce N, Guerrero E and Shah KV (1996): Male sexual behavior and human papillomavirus DNA: key risk factors for cervical cancer in Spain. J Natl Cancer Inst 88:1060–1067.

Bosch FX, Manos MM, Muñoz N, Sherman M, Jansen AM, Peto J, Schiffman MH, Moreno V, Kurman R and Shah KV (1995): Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer

(IBSCC) Study Group. J Natl Cancer Inst 87:796–802.

Bosch FX, Munoz N, de SS, Izarzugaza I, Gili M, Viladiu P, Tormo MJ, Moreo P, Ascunce N and Gonzalez LC (1992): Risk factors for cervical cancer in Colombia and Spain. Int J Cancer 52:750–758.

Bosch FX and Muñoz N (2002): The viral etiology of cervical cancer. Virus Res 89:183–190.

Bottley G, Watherston OG, Hiew YL, Norrild B, Cook GP and Blair GE (2008): High-risk human papillomavirus E7 expression reduces cell-surface MHC class I molecules and increases susceptibility to natural killer cells. Oncogene 27:1794–1799.

Bousarghin L, Touzé A, Sizaret PY and Coursaget P (2003): Human papillomavirus types 16, 31, and 58 use different endocytosis pathways to enter cells. J Virol 77:3846–3850.

Bouvard V, Matlashewski G, Gu ZM, Storey A and Banks L (1994): The human papillomavirus type 16 E5 gene cooperates with the E7 gene to stimulate proliferation of primary cells and increases viral gene expression. Virology 203:73–80.

Bramer SL and Kallungal BA (2003): Clinical considerations in study designs that use cotinine as a biomarker. Biomarkers 8:187–203.

Branca M, Duca PG, Riti MG, Rossi E, Leoncini L, Turolla E and Morosini PL (1996): Reliability and accuracy of reporting cervical intraepithelial neoplasia (CIN) in 15 laboratories throughout Italy: phase 1 of a national programme of external quality control in cervical screening. The National Working Group for External Quality Control in Cervical Screening. Cytopathology 7:159–172.

Branca M, Morosini P, Duca P, Verderio P, Giovagnoli MR, Riti MG and Leoncini L (1998): Reliability and accuracy in reporting CIN in 14 laboratories. Developing new indices of diagnostic variability in an interlaboratory study. The Working Group for External Quality Control in Cervical Cytopathology. Acta Cytol 42:1370–1376.

Breitburd F, Kirnbauer R, Hubbert NL, Nonnenmacher B, Trin-Dinh-Desmarquet C, Orth G, Schiller JT and Lowy DR (1995): Immunization with viruslike particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. J Virol 69:3959–3963.

Breslow NE and Day NE (1980): Statistical methods in cancer research. Vol. 1. The analysis of case-control studies. International Agency for Research on Cancer (IARC), Lyon: France.

Brink ATP and Peter JF (2007): Snijders and Chris J.L.M. Meijer. HPV detection methods. Disease Markers 23:273–281.

Brinton LA, Schairer C, Haenszel W, Stolley P, Lehman HF, Levine R and Savitz DA (1996): Cigarette smoking and invasive cervical cancer. JAMA 255:3265–3269.

Britton GR, Brinthaupt J, Stehle JM, James GD. Comparison of self-reported smoking and urinary cotinine levels in a rural pregnant population. J Obstet Gynecol Neonatal Nurs 2004;33:306–11.

Bulkmans NW, Berkhof J, Rozendaal L, van Kemenade FJ, Boeke AJ, Bulk S, Voorhorst FJ, Verheijen RH, van GK, Boon ME, Ruitinga W, van BM, Snijders PJ and Meijer CJ (2007): Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. Lancet 370:1764–1772.

Burd EM (2003): Human papillomavirus and cervical cancer. Clin Microbiol Rev 16:1–17.

Burkhardt A, Willingham M, Gay C, Jeang KT and Schlegel R (1989): The E5 oncoprotein of bovine papillomavirus is oriented asymmetrically in Golgi and plasma membranes. Virology 170:334–339.

Burstyn I, Kapur N, Shalapay C, Bamforth F, Wild TC, Liu J and Legatt D (2009): Evaluation of the accuracy of self-reported smoking in pregnancy when the biomarker level in an active smoker is uncertain. Nicotine Tob Res. In press.

Caberg JH, Hubert P, Herman L, Herfs M, Roncarati P, Boniver J, Delvenne P. Increased migration of Langerhans cells in response to HPV16 E6 and E7 oncogene silencing: role of CCL20. Cancer Immunol Immunother 2009;58: 39–47.

Campaner AB, Piato S, Galvão MA, dos Santos RE, Nadais RF. Langerhans cells in cervical intraepithelial neoplasia related to smoking habits. J Low Genit Tract Dis 2006;10:223–8.

Carrao MA, Guindon GE, Sharma N and Shokoohi DF (2009): Tobacco control country profiles, American Cancer Society, Atlanta, Georgia. Available at URL:http://www1.worldbank.org/tobacco /book/pdf/Introductory%20 Material.pdf.

Carter JJ, Koutsky LA, Wipf GC, Christensen ND, Lee SK, Kuypers J, Kiviat N and Galloway DA (1996): The natural history of human papillomavirus type 16 capsid antibodies among a cohort of university women. J Infect Dis 174:927–936.

Cason J, Mant CA. High-risk mucosal human papillomavirus infections during infancy & childhood (2005): J Clin Virol 32:S52–58.

Castellsague X, Bosch FX, Munoz N, Meijer CJ, Shah KV, de SS, Eluf-Neto J, Ngelangel CA, Chichareon S, Smith JS, Herrero R, Moreno V and Franceschi S (2002): Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. N Engl J Med 346:1105–1112.

Castle PE, Schiffman M and Wheeler CM (2004): Hybrid capture 2 viral load and the 2-year cumulative risk of cervical intraepithelial neoplasia grade 3 or cancer. Am J Obstet Gynecol 191:1590–1597.

Castle PE, Shields T, Kirnbauer R, Manos MM, Burk RD, Glass AG, Scott DR, Sherman ME and Schiffman M (2002): Sexual behavior, human papillomavirus type 16 (HPV 16) infection, and HPV 16 seropositivity. Sex Transm Dis 29:182–187.

Castro Felipe (2008): Human Leucocyte antigens and other genetic factors in cervical neoplasia. Doctoral dissertation, Heidelberg: Germany.

Castro FA, Haimila K, Pasanen K, Kaasila M, Patama T, Partanen J, Surcel HM, Pukkala E and Lehtinen M (2007): Geographic distribution of cervical cancer-associated human leucocyte antigens and cervical cancer incidence in Finland. Int J STD AIDS18:672–679.

Centers for Disease Control and Prevention (CDC) (2008): Human papillomavirus: HPV information for clinicians. Ala Nurse 35:17–19.

Chaouki N, Bosch FX, Muñoz N, Meijer CJ, El Gueddari B, El Ghazi A, Deacon J, Castellsagué X and Walboomers JM (1998): The viral origin of cervical cancer in Rabat, Morocco. Int J Cancer 75:546–554.

Chardonnet Y, Viac J and Thivolet J (1986): Langerhans cells in human warts. Br J Dermatol 115:669–675.

Chen EY, Howley PM, Levinson AD and Seeburg PH (1982): The primary structure and genetic organization of the bovine papillomavirus type 1 genome. Nature 299:529–534.

Chhieng DC, Talley LI, Roberson J, Gatscha RM, Jhala NC and Elgert PA (2002): Interobserver variability: comparison between liquid-based and conventional preparations in gynecologic cytology. Cancer 96:67–73.

Chiasson MA (2000): New York City AIDS case mortality rates in the era of potent antiretroviral therapy. J Urban Health 77:255–257.

Chichareon S, Herrero R, Muñoz N, Bosch FX, Jacobs MV, Deacon J, Santamaria M, Chongsuvivatwong V, Meijer CJ and Walboomers JM (1998): Risk factors for cervical cancer in Thailand: a case-control study. J Natl Cancer Inst 90:50–57.

Chua KL, Wiklund F, Lenner P, Angström T, Hallmans G, Bergman F, Sapp M, Schiller J, Wadell G, Hjerpe A and Dillner J (1996): A prospective study on the risk of cervical intra-epithelial neoplasia among healthy subjects with serum antibodies to HPV compared with HPV DNA in cervical smears. Int J Cancer 68:54–59.

Clark GC (1989): Comparison of the inhalation toxicity of kretek (clove cigarette) smoke with that of American cigarette smoke. I. One day exposure. Arch Toxicol 63:1–6.

Clarke EA, Hatcher J, McKeown-Eyssen GE and Lickrish GM (1985): Cervical dysplasia: association with sexual behavior, smoking, and oral contraceptive use? Am J Obstet Gynecol 151:612–616.

Clarke R, Shipley M, Lewington S, Youngman L, Collins R, Marmot M and Peto R (1999): Underestimations of risk associations due to regression dilution in long-term follow-up of prospective studies. Am J Epidemiol 150:341–353.

Clavel C, Masure M, Bory JP, Putaud I, Mangeonjean C, Lorenzato M, Gabriel R, Quereux C and Birembaut P (1999): Hybrid Capture II-based human papillomavirus detection, a sensitive test to detect in routine high-grade cervical lesions: a preliminary study on 1518 women. Br J Cancer 80:1306–1311.

Cogliano V, Straif K, Baan R, Grosse Y, Secretan B and El Ghissassi F (2004): Smokeless tobacco and tobacco-related nitrosamines. Lancet Oncol 5:708.

Coleman N, Birley HD, Renton AM, Hanna NF, Ryait BK, Byrne M, Taylor-Robinson D and Stanley MA (1994): Immunological events in regressing genital warts. Am J Clin Pathol 102:768–774.

Colgan TJ, McLachlin CM, Cotterchio M, Howlett R, Seidenfeld AM and Mai VM (2004): Results of the implementation of liquid-based cytology-SurePath in the Ontario screening program. Cancer 102:362–367.

Combita AL, Touzé A, Bousarghin L, Sizaret PY, Muñoz N and Coursaget P (2001): Gene transfer using human papillomavirus pseudovirions varies according to virus genotype and requires cell surface heparan sulfate. FEMS Microbiol Lett 204:183–188.

Conrad M, Bubb VJ and Schlegel R (1993): The human papillomavirus type 6 and 16 E5 proteins are membrane-associated proteins which associate with the 16-kilodalton pore-forming protein. J Virol 67:6170–6178.

Cozen W, Diaz-Sanchez D, James Gauderman W, Zadnick J, Cockburn MG, Gill PS, Masood R, Hamilton AS, Jyrala M and Mack TM (2004): Th1 and Th2 cytokines and IgE levels in identical twins with varying levels of cigarette consumption. J Clin Immunol 24:617–622.

Croucher R, Islam S, Jarvis M, Garrett M, Rahman R, Shajahan S and Howells G (2002): Tobacco dependence in a UK Bangladeshi female population: a cross-sectional study. Nicotine Tob Res 4:171–176.

Cummings SR and Richard RJ (1988): Optimum cutoff points for biochemical validation of smoking status. Am J Public Health 78:574–575.

Curado MP, Edwards BK, Shin HR, Storm H, Ferlay J, Heanue M and Boyle P (2007): Cancer Incidence in Five Continents Vol IX. Report No. 160, IARC Scientific Publication. Lyon.

Cuzick J, Sasieni P, Davies P, Adams J, Normand C, Frater A, van Ballegooijen M and van den Akker E (1999): A systematic review of the role of human papillomavirus testing within a cervical screening programme. Health Technol Assess 3:i-iv, 1–196.

Cuzick J, Sasieni P and Singer A (1996): Risk factors for invasive cervix cancer in young women. Eur J Cancer 32A:836–841.

Cuzick J (1999): Screening for cancer: future potential. Eur J Cancer 35:685–692.

Daling JR, Madeleine MM, McKnight B, Carter JJ, Wipf GC, Ashley R, Schwartz SM, Beckmann AM, Hagensee ME, Mandelson MT and Galloway DA (1996): The relationship of human papillomavirus-related cervical tumors to cigarette smoking, oral contraceptive use, and prior herpes simplex virus type 2 infection. Cancer Epidemiol Biomarkers Prev 5:541–548.

Dawar M, Deeks S and Dobson S (2007): Human papillomavirus vaccines launch a new era in cervical cancer prevention. CMAJ 177:456–461.

Davey E, Barratt A, Irwig L, Chan SF, Macaskill P, Mannes P and Saville AM (2006): Effect of study design and quality on unsatisfactory rates, cytology classifications, and accuracy in liquid-based versus conventional cervical cytology: a systematic review. Lancet 367:122–132.

de Gruijl TD, Bontkes HJ, Walboomers JM, Coursaget P, Stukart MJ, Dupuy C, Kueter E, Verheijen RH, Helmerhorst TJ, Duggan-Keen MF, Stern PL, Meijer CJ and Scheper RJ (1999): Immune responses against human papillomavirus (HPV) type 16 virus-like particles in a cohort study of women with cervical intraepithelial neoplasia. I. Differential T-helper and IgG responses in relation to HPV infection and disease outcome. J Gen Virol 80:399–408.

de Sanjose S, Bosch FX, Munoz N and Shah K (1997): Social differences in sexual behaviour and cervical cancer. IARC Sci Publ: 309–317.

de Villiers EM, Fauquet C, Broker TR, Bernard HU and zur Hausen H (2004): Classification of papillomaviruses. Virology 324:17–27.

Demeter T, Kulski JK, Sterrett GF and Pixley EC (1987): Detection of DNA of human papillomavirus types 6/11 and 16/18 in cell scrapings of the uterine cervix by filter in situ hybridisation. Correlation with cytology, colposcopy and histology. Eur J Epidemiol 3:404–413.

Denny L, Kuhn L, Pollack A and Wright TC Jr (2002): Direct visual inspection for cervical cancer screening: an analysis

of factors influencing test performance. Cancer 94:1699–1707.

Denny L (2008): Prevention of cervical cancer. Reprod Health Matters 16:18–31.

Dillner J, Kallings I, Brihmer C, Sikström B, Koskela P, Lehtinen M, Schiller JT, Sapp M and Mårdh PA (1996): Seropositivities to human papillomavirus types 16, 18, or 33 capsids and to Chlamydia trachomatis are markers of sexual behavior. J Infect Dis 173:1394–1398.

Dillner J, Lehtinen M, Björge T, Luostarinen T, Youngman L, Jellum E, Koskela P, Gislefoss RE, Hallmans G, Paavonen J, Sapp M, Schiller JT, Hakulinen T, Thoresen S and Hakama M (1997): Prospective seroepidemiologic study of human papillomavirus infection as a risk factor for invasive cervical cancer. J Natl Cancer Inst 89:1293–1299.

Dillner J (2000): Cervical cancer screening in Sweden. Eur J Cancer 36:2255–2259.

Dillner J (1999): The serological response to papillomaviruses. Semin Cancer Biol 9:423–430.

Dillner J, Rebolj M, Birembaut P, Petry KU, Szarewski A, Munk C, de Sanjosé S, Naucler P, Lloveras B, Kjaer S, Cuzick J, van Ballegooijen M, Clavel C and Iftner T (2008): Long term predictive values of cytology and human papillomavirus testing in cervical cancer screening: joint European cohort study. BMJ 337: a1754.

Doll R, Payne P and Waterhouse J (eds) (1996): Cancer Incidence in Five Continents Vol I. UICC Geneva, Springer- Verlag, Berlin.

Doll R, Peto R, Boreham J and Sutherland I (2004): Mortality in relation to smoking: 50 years' observations on male British doctors. BMJ 328:1519–1528.

Doorbar J, Ely S, Sterling J, McLean C and Crawford L (1991):. Specific interaction between HPV-16 E1-E4 and cytokeratins results in collapse of the epithelial cell intermediate filament network. Nature 352:824–827.

Doorbar J, Medcalf E and Napthine S (1996): Analysis of HPV1 E4 complexes and their association with keratins in vivo. Virology 218:114–126.

Doorbar J (2006): Molecular biology of human papillomavirus infection and cervical cancer. Clin Sci (Lond) 110:525–541.

Doorbar J (2007): Papillomavirus life cycle organization and biomarker selection. Dis Markers. 23:297–313.

Dorn FH and Cutler JS (1959): Morbidity from cancer in the United States. Public Health Monogr 73:1–207.

Dowie R, Stoykova B, Crawford D, Desai M, Mather J, Morgan K and Shirt M (2006): Liquid-based cytology can improve efficiency of cervical smear readers: evidence from timing surveys in two NHS cytology laboratories. Cytopathology17:65–72.

Doyle B, O'Farrell C, Mahoney E, Turner L, Magee D and Gibbons D (2006): Liquid-based cytology improves productivity in cervical cytology screening. Cytopathology 17:60–64.

Eliopoulos C, Klein J and Koren G (1996): Validation of self-reported smoking by analysis of hair for nicotine and cotinine. Therapeutic Drug Monitoring 18:532–536.

El-Sherif AM, Seth R, Tighe PJ and Jenkins D (2001): Quantitative analysis of IL-10 and IFN-gamma mRNA levels in normal cervix and human papillomavirus type 16 associated cervical precancer. J Pathol 195:179–185.

Eluf-Neto J, Booth M, Muñoz N, Bosch FX, Meijer CJ and Walboomers JM (1994): Human papillomavirus and invasive cervical cancer in Brazil. Br J Cancer 69:114–119.

Engeland A, Haldorsen T and Tretli S (1995): Prediction of cancer incidence in the Nordic countries up to the year 2000 and 2010: a collaborative study of the five Nordic cancer registries. APMIS suppl 49:1–161.

Evander M, Frazer IH, Payne E, Qi YM, Hengst K and McMillan NA (1997): Identification of the alpha6 integrin as a candidate receptor for papillomaviruses. J Virol 71:2449–2456.

Fairley CK, Sheil AG, McNeil JJ, Ugoni AM, Disney AP, Giles GG and Amiss N (1994): The risk of ano-genital malignancies in dialysis and transplant patients. Clin Nephrol 41:101–105.

Fehrmann F, Klumpp DJ and Laimins LA (2003): Human papillomavirus type 31 E5 protein supports cell cycle progression and activates late viral functions upon epithelial differentiation. J Virol 77:2819–2831.

Fehrmann F and Laimins LA (2003): Human papillomaviruses: targeting differentiating epithelial cells for malignant transformation. Oncogene 22:5201–5207.

Fenercioglu AK, Tamer I, Karatekin G, Nuhoglu A (2009): Impaired postnatal growth of infants prenatally exposed to cigarette smoking.Tohoku J Exp Med 218: 221–228.

Ferenczy A and Franco E (2001): Cervical-cancer screening beyond the year 2000. Lancet Oncol: 27–32.

Ferlay J, Bray F, Pisani P and Parkin DM (2004) GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide. IARC cancer-base No. 5 version 2.0. IARC Press, Lyon.

Ferrera A, Baay MF, Herbrink P, Figueroa M, Velema JP ans Melchers WJ (1997): A seroepidemiological study of the relationship between sexually transmitted agents and cervical cancer in Honduras. Int J Cancer 73:781–785.

Feyerabend C and Russell MAH (1990): A rapid gas-liquid chromatographic method for the determination of cotinine and nicotine in biological fluids. J Pharm Pharmacol 42:450–452.

Franceschi S, Rajkumar T, Vaccarella S, Gajalakshmi V, Sharmila A, Snijders PJ, Munoz N, Meijer CJ and Herrero R (2003): Human papillomavirus and risk factors for cervical cancer in Chennai, India: a case-control study. Int J Cancer 107:127–133.

Franco EL (2003): Chapter 13: Primary screening of cervical cancer with human papillomavirus tests. J Natl Cancer Inst Monogr: 89–96.

Frazer IH (2009): Interaction of human papillomaviruses with the host immune system: a well evolved relationship. Virology 384:410–414.

Frazer IH (2004): Prevention of cervical cancer through papillomavirus vaccination. Nat Rev Immunol 4:46–54.

Gajalakshmi V, Peto R, Kanaka TS and Jha P (2003): Smoking and mortality from tuberculosis and other diseases in India: retrospective study of 43,000 adult male deaths and 35,000 controls. Lancet 362:507–515.

Gan CY (1995): Smokeless tobacco use among rural Kadazan women in Sabah, Malaysia. Southeast Asian J Trop Med Public Health 26:291–296.

Garcia-Closas R, Castellsague X, Bosch X and Gonzalez CA (2005): The role of diet and nutrition in cervical carcinogenesis: a review of recent evidence. Int J Cancer 117:629–637.

Giannini SL, Hanon E, Moris P, Van Mechelen M, Morel S, Dessy F, Fourneau MA, Colau B, Suzich J, Losonksy G, Martin MT, Dubin G and Wettendorff MA (2006): Enhanced humoral and memory B cellular immunity using HPV16/18 L1 VLP vaccine formulated with the MPL/aluminium salt combination (AS04) compared to aluminium salt only. Vaccine 24:5937–5949.

Gillison ML and Shah KV (2003): Chapter 9: Role of mucosal human papillomavirus in nongenital cancers. J Natl Cancer Inst Monogr: 57–65.

Giroglou T, Florin L, Schäfer F, Streeck RE and Sapp M (2001): Human papillomavirus infection requires cell surface heparan sulfate. J Virol 75:1565–1570.

Giuliano AR, Lazcano-Ponce E, Villa L, Nolan T, Marchant C, Radley D, Golm G, McCarroll K, Yu J, Esser MT, Vuocolo SC and Barr E (2007):. Impact of baseline covariates on the immunogenicity of a quadrivalent (types 6, 11, 16, and 18) human papillomavirus virus-like-particle vaccine. J Infect Dis 196:1153–1162.

Global Youth Tobacco Survey (GYTS) Collaborating Group (2003): Differences in worldwide tobacco use by gender: findings from the Global Youth Tobacco Survey. J Sch Health 73:207–15.

Global Youth Tobacco Survey (GYTS) Collaboration Group (2002): Tobacco use among youth: a cross country comparison. Tob Control 11:252–270.

Gonçalves MA and Donadi EA (2004): Immune cellular response to HPV: current concepts. Braz J Infect Dis 8:1–9.

Goodman J (1995): Tobacco in history: The cultures of dependence. Routledge London and New York.

Grassmann K, Rapp B, Maschek H, Petry KU and Iftner T (1996): Identification of a differentiation-inducible promoter in the E7 open reading frame of human papillomavirus type 16 (HPV-16) in raft cultures of a new cell line containing high copy numbers of episomal HPV-16 DNA. J Virol 70:2339–2349.

Gravitt PE, Peyton CL, Apple RJ and Wheeler CM (1998): Genotyping of 27 human papillomavirus types by using L1 consensus products by a single-hybridization, reverse line blot detection method. J Clin Microbiol 36:3020–3027.

Guess JC and McCance DJ (2005): Decreased migration of Langerhans precursor-like cells in response to human keratinocytes expressing human papillomavirus type 16 E6/E7 is related to reduced

macrophage inflammatory protein-3alpha production. J Virol 79:14852–14862.

Guindon GE and Boisclair D (2003):. Past, current and future trends in tobacco use. Economics of Tobacco control, paper No 6. Health, Nutrition and Population discussion paper.

Gunnell AS, Tran TN, Torrång A, Dickman PW, Sparén P, Palmgren J, Ylitalo N(1999):. Synergy between cigarette smoking and human papillomavirus type 16 in cervical cancer in situ development. Cancer Epidemiol Biomarkers Prev 2006;15:2141–7.

Hakulinen T, Hörte LG, Luostarinen T, Schou G, Sigvaldason H, Storm HH and Tulinius H (1995): Prediction of cancer incidence in the Nordic countries up to the year 2000 and 2010: a collaborative study of the five Nordic cancer registries. APMIS suppl 49:1–161.

Hamid NA, Brown C and Gaston K (2009): The regulation of cell proliferation by the papillomavirus early proteins. Cell Mol Life Sci. In press.

Hammouda D, Munoz N, Herrero R, Arslan A, Bouhadef A, Oublil M, Djedeat B, Fontaniere B, Snijders P, Meijer C and Franceschi S (2005): Cervical carcinoma in Algiers, Algeria: human papillomavirus and lifestyle risk factors. Int J Cancer 113:483–89.

Handsfield HH (1997): Sex, science, and society. A look at sexually transmitted diseases. Postgrad Med 101:268-73, 277–8.

Hanna L and Keshishyane H (1980): Chlamydial antigens stabilized with formalin for use in the microimmunofluorescence test. J Clin Microbiol 12:409.

Harper DM and Dubin G (2007): Sustained HPV-16 and 18 antibody levels through 5.5 years in women vaccinated with the HPV16/18 L1 VLP AS04 candidate vaccine (abstract). The HPV vaccine study group. 15th International Meeting for the European Society for Gynaecological Oncology (ESGO); Berlin. Available from URL: http://www.multiwebcast.comesgo/2007/15th. Accessed 2009 April 28.

Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A, Zahaf T, Innis B, Naud P, De Carvalho NS, Roteli-Martins CM, Teixeira J, Blatter MM, Korn AP, Quint W and Dubin G (2004): Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. Lancet 364:1757–1765.

Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM, Jenkins D, Schuind A, Costa Clemens SA and Dubin G (2006): Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. Lancet 367:1247–1255.

Haufroid V and Lison D (1998): Urinary cotinine as a tobacco-smoke exposure index: A minireview. International Archives of Occupational and Environmental Health 71:162–168.

Hawes SE, Critchlow CW, Sow PS, Toure P, N'Doye I, Diop A, Kuypers JM, Kasse AA and Kiviat NB(2006): Incident high-grade squamous intraepithelial lesions in Senegalese women with and without human immunodeficiency virus type 1 (HIV-1) and HIV2. J Natl Cancer Inst 98:100–109.

Hawley-Nelson P, Vousden KH, Hubbert NL, Lowy DR and Schiller JT (1989): HPV16 E6 and E7 proteins cooperate to immortalize human foreskin keratinocytes. EMBO J. 8:3905–3910.

Hebner CM and Laimins LA (2006): Human papillomaviruses: basic mechanisms of pathogenesis and oncogenicity. Rev Med Virol 16:83–97.

Helakorpi S, Martelin T, Torppa J, Patja K, Vartiainen E and Uutela A (2004): Did Finland's Tobacco Control Act of 1976 has an impact on ever smoking? An examination based on male and female cohort trends. J Epidemiol Community Health 58:649–654.

Helakorpi S, Patja K, Prattala R and Uutela A (2005): Health Behaviour and Health among the Finnish Adult Population, Spring 2005. Publications of the National Institute for Health and Welfare B18/2005.

Hesselink AT, van den Brule AJ and Groothuismink ZM (2005): Comparison of three different PCR methods for quantifying human papillomavirus type 16 DNA in cervical scrape specimens. J Clin Microbiol 43:4868–4871.

Hildesheim A, Herrero R, Castle PE, Wacholder S, Bratti MC, Sherman ME, Lorincz AT, Burk RD, Morales J, Rodriguez AC, Helgesen K, Alfaro M, Hutchinson M, Balmaceda I, Greenberg M and Schiffman M (2001): HPV co-

factors related to the development of cervical cancer: results from a population-based study in Costa Rica. Br J Cancer 84:1219–1226.

Hildesheim A, Herrero R, Wacholder S, Rodriguez AC, Solomon D, Bratti MC, Schiller JT, Gonzalez P, Dubin G, Porras C, Jimenez SE and Lowy DR (2007): Costa Rican HPV Vaccine Trial Group. Effect of human papillomavirus 16/18 L1 viruslike particle vaccine among young women with preexisting infection: a randomized trial. JAMA 298:743–753.

Hildesheim A, Mann V, Brinton LA, Szklo M, Reeves WC and Rawls WE (1991): Herpes simplex virus type 2: a possible interaction with human papillomavirus types 16/18 in the development of invasive cervical cancer. Int J Cancer 49:335–340.

Hirose K, Tajima K, Hamajima N, Takezaki T, Inoue M, Kuroishi T, Kuzuya K, Nakamura S and Tokudome S (1996): Subsite (cervix/endometrium)-specific risk and protective factors in uterus cancer. Jpn J Cancer Res 87:1001–1009.

Ho GY, Kadish AS, Burk RD, Basu J, Palan PR, Mikhail M and Romney SL (1998): HPV 16 and cigarette smoking as risk factors for high-grade cervical intra-epithelial neoplasia. Int J Cancer 78:281–285.

Hoffmann D and Djordjevic MV (1997): Chemical composition and carcinogenicity of smokeless tobacco. Adv Dent Res 11:322–329.

Holly EA, Cress RD, Ahn DK, Aston DA, Kristiansen JJ, Wu R and Felton JS (1993): Detection of mutagens in cervical mucus in smokers and nonsmokers. Cancer Epidemiol Biomarkers Prev 2:223–228.

Holowaty P, Miller AB, Rohan T and To T (1999): Natural history of dysplasia of the uterine cervix. J Natl Cancer Inst 91:252–258.

Horner SM and DiMaio D (2007): The DNA binding domain of a papillomavirus E2 protein programs a chimeric nuclease to cleave integrated human papillomavirus DNA in HeLa cervical carcinoma cells. J Virol 81:6254–6264.

Howley PM, Spehner D and Drillien R (1996): A vaccinia virus transfer vector using a GUS reporter gene inserted into the I4L locus. Gene 172:233–237.

Howley PM (2006): Warts, cancer and ubiquitylation: lessons from the papillomaviruses. Trans Am Clin Climatol Assoc117:113–126.

Hussain SK, Madeleine MM, Johnson LG, Du Q, Malkki M, Wilkerson HW, Farin FM, Carter JJ, Galloway DA, Daling JR, Petersdorf EW and Schwartz SM (2008): Cervical and vulvar cancer risk in relation to the joint effects of cigarette smoking and genetic variation in interleukin 2. Cancer Epidemiol Biomarkers Prev 17:1790–1799.

Häfner N, Gajda M, Altgassen C, Hertel H, Greinke C, Hillemanns P, Schneider A and Dürst M (2007): HPV16-E6 mRNA is superior to cytokeratin 19 mRNA as a molecular marker for the detection of disseminated tumour cells in sentinel lymph nodes of patients with cervical cancer by quantitative reverse-transcription PCR. Int J Cancer 120:1842–1846.

International Agency for Research on Cancer (IARC) (2005): IARC Handbooks of Cancer Prevention. Volume 10: Cervix Cancer Screening, IARCPress, Lyon, France.

International Agency for Research on Cancer (IARC) (2004a): IARC Handbooks on Cancer Prevention. Volume 10: Cervix cancer screening, IARCPress, Lyon, France.

International Agency for Research on Cancer (IARC) (2006): IARC monographs on evaluation of the carcinogenic risks to humans: Human Papillomaviruses. IARCPress, Lyon, France.

International Agency for Research on Cancer (IARC) (2004b): IARC Monographs on the evaluation of the carcinogenic risk of chemical to human. Vol 83. Tobacco smoking and unvoluntary smoke. IARCPress Lyon, France.

International Agency for Research on Cancer (IARC) (1986): IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemichals to Humans. Vol 38. Tobacco Smoking. IARCPress, Lyon France.

Idle JR (1990): Titrating exposure to tobacco smoke using cotinine—a minefield of misunderstandings. J Clin Epidemiol 43:313–317.

International correlation between human papillomavirus prevalence and cervical cancer incidence. Jacob P, 3rd, Yu L, Shulgin AT and Benowitz NL (1999): Minor tobacco alkaloids as biomarkers for tobacco use: comparison of users of cigarettes, smokeless tobacco, cigars, and pipes. Am J Public Health 89:731–736.

James H, Tizabi Y and Taylor R (1998): Rapid method for the simultaneous measurement of nicotine and cotinine in urine and serum by gas chromatography–mass spectrometry. Journal of Chromatography B: Biomedical Sciences and Applications 708:87–93.

Jansen KU, Rosolowsky M, Schultz LD, Markus HZ, Cook JC, Donnelly JJ, Martinez D, Ellis RW and Shaw AR (1995): Vaccination with yeast-expressed cottontail rabbit papillomavirus (CRPV) virus-like particles protects rabbits from CRPV-induced papilloma formation. Vaccine 13:1509–1514.

Jarvis MJ, Tunstall-Pedoe H, Feyerabend C, Vesey C and Saloojee Y (1987): Comparison of tests used to distinguish smokers from nonsmokers. Am J Public Health 77:1435–1438.

Josefsson A, Livak K and Gyllensten U (1999): Detection and quantitation of human papillomavirus by using the fluorescent 5'exonuclease assay. J Clin Microbiol 37:490–496.

Josefsson AM, Magnusson PK, Ylitalo N, Sørensen P, Qwarforth-Tubbin P, Andersen PK, Melbye M, Adami HO and Gyllensten UB (2000): Viral load of human papilloma virus 16 as a determinant for development of cervical carcinoma in situ: a nested case-control study. Lancet 355:2189–2193.

Joyce JG, Tung JS, Przysiecki CT, Cook JC, Lehman ED, Sands JA, Jansen KU ans Keller PM (1999): The L1 major capsid protein of human papillomavirus type 11 recombinant virus-like particles interacts with heparin and cell-surface glycosaminoglycans on human keratinocytes. J Biol Chem 274:5810–5822.

Kadish AS, Ho GY, Burk RD, Wang Y, Romney SL, Ledwidge R and Angeletti RH (1997): Lymphoproliferative responses to human papillomavirus (HPV) type 16 proteins E6 and E7: outcome of HPV infection and associated neoplasia. J Natl Cancer Inst 89:1285–1293.

Kanodia S, Fahey LM and Kast WM (2007): Mechanisms used by human papillomaviruses to escape the host immune response. Curr Cancer Drug Targets 7:79–89.

Kataja V, Syrjänen S, Mäntyjärvi R, Yliskoski M, Saarikoski S and Syrjänen K (1992): Prognostic Factors in Cervical Human Papillomavirus Infections. Sex Transm Dis 19:154–160.

Kathleen RS and Muñoz LR (2004): Cigarette smoking: Evidence to guide measurement. Research in Nursing and Health 27:281–292.

Khan AM and Singer A (2008): Biomarkers in cervical precancer management: the new frontiers. Future Oncol 4:515–524.

Kim D, Monie A, He L, Tsai YC, Hung CF and Wu TC (2008): Role of IL-2 secreted by PADRE-specific CD4+ T cells in enhancing E7-specific CD8+ T-cell immune responses. Gene Ther 15:677–687.

Kirnbauer R, Booy F, Cheng N, Lowy DR and Schiller JT (1992): Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. Proc Natl Acad Sci U S A 89:12180–12184.

Kirnbauer R, Hubbert NL, Wheeler CM, Becker TM, Lowy DR and Schiller JT (1994): A virus-like particle enzyme-linked immunosorbent assay detects serum antibodies in a majority of women infected with human papillomavirus type 16. J Natl Cancer Inst 86:494–499.

Kjaer SK, Dahl C, Engholm G, Bock JE, Lynge E and Jensen OM (1992): Case-control study of risk factors for cervical neoplasia in Denmark. II. Role of sexual activity, reproductive factors, and venereal infections. Cancer Causes Control 3:339–348.

Kjellberg L, Wadell G, Bergman F, Isaksson M, Angstrom T and Dillner J (2000): Regular disappearance of the human papillomavirus genome after conization of cervical dysplasia by carbon dioxide laser. Am J Obstet Gynecol 183:1238–1242.

Kjellberg L, Wang Z, Wiklund F, Edlund K, Angström T, Lenner P, Sjöberg I, Hallmans G, Wallin KL, Sapp M, Schiller J, Wadell G, Mählck CG and Dillner J (1999): Sexual behaviour and papillomavirus exposure in cervical intraepithelial neoplasia: a population-based case-control study. J Gen Virol 80: 391–398.

Kleter B, van Doorn LJ, Schrauwen L, Molijn A, Sastrowijoto S, ter Schegget J, Lindeman J, ter Harmsel B, Burger M and Quint W (1999): Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. J Clin Microbiol 37:2508–2517.

Knight GJ, Wylie P, Holman MS and Haddow JE (1985): Improved ^{125}I radioimmunoassay for cotinine by selective removal of bridge antibodies. Clin Chem 31:118–121.

Knight GL, Grainger JR, Gallimore PH and Roberts S (2004): Cooperation between different forms of the human papillomavirus type 1 E4 protein to block cell cycle progression and cellular DNA synthesis. J Virol 78:13920–13933.

Koliopoulos G, Arbyn M, Martin-Hirsch P, Kyrgiou M, Prendiville W and Paraskevaidis E (2007): Diagnostic accuracy of human papillomavirus testing in primary cervical screening: a systematic review and meta-analysis of non-randomized studies. Gynecol Oncol 104:232–246.

Kónya J, Eklund C, af Geijersstam V, Yuan F, Stuber G and Dillner J (1997): Identification of a cytotoxic T-lymphocyte epitope in the human papillomavirus type 16 E2 protein. J Gen Virol 78 (Pt 10):2615–2620.

Kordi Tamandani MK, Sobti RC, Shekari M, Mukesh M and Suri V (2008): Expression and polimorphism of IFN-gamma gene in patients with cervical cancer. Exp Oncol 30:224–229.

Korhonen T, Urjanheimo EL, Mannonen P, Korhonen HJ, Uutela A and Puska P (1999): Quit and Win campaigns as a long-term anti-smoking intervention in North Karelia and other parts of Finland. Tob Control 8:175–181.

Koskela P, Anttila T, Bjørge T, Brunsvig A, Dillner J, Hakama M, Hakulinen T, Jellum E, Lehtinen M, Lenner P, Luostarinen T, Pukkala E, Saikku P, Thoresen S, Youngman L and Paavonen J (2000): Chlamydia trachomatis infection as a risk factor for invasive cervical cancer. Int J Cancer 85:35–39.

Kotaniemi-Talonen L, Malila N, Nieminen P, Anttila A, Tarkkanen J, Laurila P and Hakama M (2008): Test positivity cutoff level of a high risk human papillomavirus test could be increased in routine cervical cancer screening. Int J Cancer 123:2902–2906.

Kotaniemi-Talonen L, Nieminen P, Hakama M, Seppänen J, Ikkala J, Martikainen J, Tarkkanen J, Toivonen T and Anttila A (2007): Significant variation in performance does not reflect the effectiveness of the cervical cancer screening programme in Finland. Eur J Cancer 43:169–174.

Kotianemi-Talonen L, Nieminen P, Antilla A and Hakama M (2005): Routine cervical screening with primary HPV testing and cytology triage protocol in a randomised setting. BJC 93:862–867.

Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, Chiacchierini LM and Jansen KU (2002): A controlled trial of a human papillomavirus type 16 vaccine. N Engl J Med 347:1645–1651.

Koutsky LA and Harper DM (2006): Chapter 13: Current findings from prophylactic HPV vaccine trials. Vaccine 24 Suppl 3: S114–S121.

Kulmala SM, Syrjänen S, Shabalova I, Petrovichev N, Kozachenko V, Podistov J, Ivanchenko O, Zakharenko S, Nerovjna R, Kljukina L, Branovskaja M, Grunberga V, Juschenko A, Tosi P, Santopietro R and Syrjänen K (2004): Human papillomavirus testing with the hybrid capture 2 assay and PCR as screening tools. J Clin Microbiol 42:2470–2475.

La Ruche G, Ramon R, Mensah-Ado I, Bergeron C, Diomandé M, Sylla-Koko F, Ehouman A, Touré-Coulibaly K, Welffens-Ekra C and Dabis F (1998): Squamous intraepithelial lesions of the cervix, invasive cervical carcinoma, and immunosuppression induced by human immunodeficiency virus in Africa. Dyscer-CI Group. Cancer 82:2401–2408.

La Vecchia C, Franceschi S, Decarli A, Fasoli M, Gentile A and Tognoni G (1986): Cigarette smoking and the risk of cervical neoplasia. Am J Epidemiol 123:22–29.

Lambert PF (1991): Papillomavirus DNA replication. J Virol 65:3417–3420.

Lando HA, McGovern PG, Kelder SH, Jeffery RW and Forster JL (1991): Use of carbon monoxide breath validation in assessing exposure to cigarette smoke in a worksite population. Health Psychology 10:296–301.

Laukkanen P, Koskela P, Pukkala E, Dillner J, Läärä E, Knekt P and Lehtinen M (2003): Time trends in incidence and prevalence of human papillomavirus type 6, 11 and 16 infections in Finland. J Gen Virol 84:2105–2109.

Le Cann P, Touze A, Leboulleux D, Mougin C, Legrand MC, Calvet C, Afoutou JM and Coursaget P (1995): Detection of antibodies against human papillomavirus (HPV) type 16 virions by enzyme-linked immunosorbent assay using recombinant HPV 16 L1 capsids

produced by recombinant baculovirus. J Clin Microbiol 33:1380–1382.

Lehtinen M, Apter D, Dubin G, Kosunen E, Isaksson R, Korpivaara EL, Kyhä-Osterlund L, Lunnas T, Luostarinen T, Niemi L, Palmroth J, Petäjä T, Rekonen S, Salmivesi S, Siitari-Mattila M, Svartsjö S, Tuomivaara L, Vilkki M, Pukkala E and Paavonen J (2006a): Enrolment of 22,000 adolescent women to cancer registry follow-up for long-term human papillomavirus vaccine efficacy: guarding against guessing. Int J STD AIDS 17:517–521.

Lehtinen M, Dillner J, Knekt P, Luostarinen T, Aromaa A, Kirnbauer R, Koskela P, Paavonen J, Peto R, Schiller JT and Hakama M (1996): Serologically diagnosed infection with human papillomavirus type 16 and risk for subsequent development of cervical carcinoma: nested case-control study. BMJ 312:537–539.

Lehtinen M, Kaasila M, Pasanen K, Toni P, Palmroth J, Laukkanen P, Pukkala E and Koskela P (2006b): Seroprevalence atlas of infections with oncogenic and non-oncogenic human papillomaviruses in Finland in the 1980s and 1990s. Int J Cancer 119:2612–2619.

Lehtinen M, Koskela P, Jellum E, Bloigu A, Anttila T, Hallmans G, Luukkaala T, Thoresen S, Youngman L, Dillner J and Hakama M (2002): Herpes simplex virus and risk of cervical cancer: a longitudinal, nested case-control study in the Nordic countries. Am J Epidemiol 156:687–692.

Lehtinen M, Leminen A, Paavonen J, Lehtovirta P, Hyöty H, Vesterinen E and Dillner J (1992): Predominance of serum antibodies to synthetic peptide stemming from HPV 18 open reading frame E2 in cervical adenocarcinoma. J Clin Pathol 45:494–497.

Lehtinen M, Luukkaala T, Wallin KL, Paavonen J, Thoresen S, Dillner J and Hakama M (2001): Human papillomavirus type 16 infection and risk for subsequent development of cervical neoplasia—a systematic review. J Clin Virol 19:537–539.

Lehtinen M, Pawlita M, Zumbach K, Lie K, Hakama M, Jellum E, Koskela P, Luostarinen T, Paavonen J, Pukkala E, Sigstad E, Thoresen S and Dillner J (2003): Evaluation of antibody response to human papillomavirus early proteins in women in whom cervical cancer

developed 1 to 20 years later. Am J Obstet Gynecol 188:49–55.

Leminen A, Paavonen j, Vesterinen E, Wahlstrom T, Rantala I and Lehtinen M (1992): Human papillomavirus types 16 and 18 in adenocarcinoma of the uterine cervix. Am J Clin Pathol 95:647–652.

Leyden WA, Manos MM, Geiger AM, Weinmann S, Mouchawar J, Bischoff K, Yood MU, Gilbert J and Taplin SH (2005): Cervical cancer in women with comprehensive health care access: attributable factors in the screening process. J Natl Cancer Inst 97:675–683.

Li M, Davey GM, Sutherland RM, Kurts C, Lew AM, Hirst C, Carbone FR and Heath WR (2001): Cell-associated ovalbumin is cross-presented much more efficiently than soluble ovalbumin in vivo. J Immunol 166:6099–6103.

Liu BQ, Peto R, Chen ZM, Boreham J, Wu YP, Li JY, Campbell TC and Chen JS (1998): Emerging tobacco hazards in China: 1. Retrospective proportional mortality study of one million deaths. BMJ 317:1411–1422.

Liu DW, Yang YC, Lin HF, Lin MF, Cheng YW, Chu CC, Tsao YP and Chen SL (2007): Cytotoxic T-lymphocyte responses to human papillomavirus type 16 E5 and E7 proteins and HLA-A*0201-restricted T-cell peptides in cervical cancer patients. J Virol 81:2869–2879.

Mackay J (2001): Preface. In J.M. Samet & S.Y. Yoon (Eds.). *Women and the tobacco epidemic -- Challenges for the 21st century*. Geneva: World Health Organization.

Mackey J and Ericksen M (2003): The Tobacco Atlas, Geneva, World Health Organization.

Madeleine MM, Anttila T, Schwartz SM, Saikku P, Leinonen M, Carter JJ, Wurscher M, Johnson LG, Galloway DA and Daling JR (2007): Risk of cervical cancer associated with Chlamydia trachomatis antibodies by histology, HPV type and HPV cofactors. Int J Cancer 120:650–655.

Magnusson PK, Sparén P and Gyllensten UB (1999): Genetic link to cervical tumours. Nature 400:29–30.

Malson JL, Lee EM, Moolchan ET and Pickworth WB (2001): Nicotine delivery from smoking bidis and an additive-free cigarette. Nicotine Tob Res 4:485–490.

Malson JL and Pickworth WB (2002): Bidis – Hand-rolled, Indian cigarettes: effects on physical, biochemical and subjective

measures. Pharmacol Biochem Behav 72:443–447.

Maucort-Boulch D, Franceschi S and Plummer M (2008): IARC HPV Prevalence Surveys Study Group. Cancer Epidemiol Biomarkers Prev 17:717–720.

Mayrand MH, Duarte-Franco E, Rodrigues I, Walter SD, Hanley J, Ferenczy A, Ratnam S, Coutlee F and Franco EL (2007): Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. N Engl J Med 357:1579–1588.

McIntyre-Seltman K, Castle PE, Guido R, Schiffman M and Willer CM (2005): ALTS Group. Smoking is a risk factor for cervical intraepithelial neoplasia grade 3 among oncogenic human papillomavirus DNA-positive women with equivocal or mildly abnormal cytology. Cancer Epidemiol Biomarkers Prev 14:1165–1170.

Mehta FS, Pindborg JJ, Gupta PC and Daftary DK (1969): Epidemiology and history study of oral cancer and leukoplakia among 101,761 villagers in India. Cancer 24:832–849.

Melikian AA, Sun P, Prokopczyk B, El-Bayoumy K, Hoffmann D, Wang X and Waggoner S (1999): Identification of benzo[a]pyrene metabolites in cervical mucus and DNA adducts in cervical tissues in humans by gas chromatography-mass spectrometry. Cancer Lett 146:127–134.

Melnikow J, Nuovo J, Willan AR, Chan BK and Howell LP (1998): Natural history of cervical squamous intraepithelial lesions: a meta-analysis. Obstet Gynecol 92:727–735.

Meyer S, Raisig A, Gortner L, Ong MF, Bücheler M, Tutdibi E (2009): In utero tobacco exposure: The effects of heavy and very heavy smoking on the rate of SGA infants in the Federal State of Saarland, Germany. Eur J Obstet Gynecol Reprod Biol (in press).

Mitchell MF, Hittelman WN, Hong WK, Lotan R and Schottenfeld D (1994): The natural history of cervical intraepithelial neoplasia: an argument for intermediate endpoint biomarkers. Cancer Epidemiol Biomarkers Prev 3:619–626.

Moberg M, Gustavsson I and Gyllensten U (2003): Real-time PCR-based system for simultaneous quantification of human papillomavirus types associated with high risk of cervical cancer. J Clin Microbiol 41:3221–3228.

Molarius A, Parsons RW, Dobson AJ, Evans A, Fortmann SP, Jamrozik K, Kuulasmaa K, Moltchanov V, Sans S, Tuomilehto J and Puska P (2001): WHO MONICA Project. Trends in cigarette smoking in 36 populations from the early 1980s to the mid-1990s: findings from the WHO MONICA Project. Am J Public Health 91:206–212.

Moodley M (2005): Update on pathophysiologic mechanisms of human papillomavirus. Curr Opin Obstet Gynecol 17:61Y4.

Mork J, Lie AK, Glattre E, Hallmans G, Jellum E, Koskela P, Møller B, Pukkala E, Schiller JT, Youngman L, Lehtinen M and Dillner J (2001): Human papillomavirus infection as a risk factor for squamous cell carcinoma of the head and neck. New Eng J Med 344:1125–1131.

Moscicki AB, Hills N, Shiboski S, Powell K, Jay N, Hanson E, Miller S, Clayton L, Farhat S, Broering J, Darragh T and Palefsky J (2001): Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. JAMA 285:2995–3002.

Motoyama S, Ladines-Llave CA, Luis Villanueva S and Maruo T (2004): The role of human papilloma virus in the molecular biology of cervical carcinogenesis. Kobe J Med Sci 50:9–19.

Muñoz N, Bosch FX, Castellsagué X, Diaz M, de Sanjosé S, Hammouda D, Shah KV and Meijer CJ (2004): Against which human papillomavirus types shall we vaccinate and screen? The international perspective. Int J Cancer 111:278–285.

Muñoz N, Bosch FX, de Sanjosé S, Vergara A, del Moral A, Muñoz MT, Tafur L, Gili M, Izarzugaza I and Viladiu P (1993): Risk factors for cervical intraepithelial neoplasia grade III/carcinoma in situ in Spain and Colombia. Cancer Epidemiol Biomarkers Prev 2:423–431.

Murray RP, Connett JE, Istvan JA, Nides MA and Rempel-Rossum S (2002): Relations of cotinine and carbon monoxide to self-reported smoking in a cohort of smokers and ex-smokers followed over 5 years. Nicotine Tob Res 4:287–294.

Murray RP, Connett JE, Lauger GG and Voelker HT (1993): Error in smoking measures: effects of intervention on relations of cotinine and carbon monoxide to self-reported smoking. The Lung Health Study Research Group. Am J Public Health 83:1251–1257.

Muwonge R, Ramadas K, Sankila R, Thara S, Thomas G, Vinoda J and Sankaranarayanan R (2008): Role of tobacco smoking, chewing and alcohol drinking in the risk of oral cancer in Trivandrum, India: A nested case-control design using incident cancer cases. Oral Oncol 44:446–454.

Müller M, Gissmann L, Cristiano RJ, Sun XY, Frazer IH, Jenson AB, Alonso A, Zentgraf H and Zhou J (1995): Papillomavirus capsid binding and uptake by cells from different tissues and species. J Virol 69:948–954.

Nakagawa M, Stites DP, Patel S, Farhat S, Scott M, Hills NK, Palefsky JM and Moscicki AB (2000): Persistence of human papillomavirus type 16 infection is associated with lack of cytotoxic T lymphocyte response to the E6 antigens. J Infect Dis 182:595–598.

Nakamura Y, Miyata M, Ohba T, Ando T, Hatsushika K, Suenaga F, Shimokawa N, Ohnuma Y, Katoh R, Ogawa H and Nakao A (2008): Cigarette smoke extract induces thymic stromal lymphopoietin expression, leading to T(H)2-type immune responses and airway inflammation. J Allergy Clin Immunol 122:1208–1214.

Nanda K, McCrory DC, Myers ER, Bastian LA, Hasselblad V, Hickey JD and Matchar DB (2000): Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. Ann Intern Med 132:810–819.

Nandakumar A, Gupta PC, Gangadharan P, Visweswara RN and Parkin DM (2005): Geographic pathology revisited: development of an atlas of cancer in India. Int J Cancer 116:740–754.

Nardelli-Haefliger D, Wirthner D, Schiller JT, Lowy DR, Hildesheim A, Ponci F and De Grandi P (2003): Specific antibody levels at the cervix during the menstrual cycle of women vaccinated with human papillomavirus 16 virus-like particles. J Natl Cancer Inst 95:1128–1137.

National Infectious Diseases Register (NIDR) (2007): National Institute for Health and Welfare (THL), Helsinki, Finland.

Naucler P, Ryd W, Tornberg S, Strand A, Wadell G, Elfgren K, Radberg T, Strander B, Forslund O, Hansson BG, Rylander E and Dillner J (2007): Human papillomavirus and Papanicolaou tests to screen for cervical cancer. N Engl J Med 357:1589–1597.

Ngelangel C, Muñoz N, Bosch FX, Limson GM, Festin MR, Deacon J, Jacobs MV, Santamaria M, Meijer CJ and Walboomers JM (1998):. Causes of cervical cancer in the Philippines: a case-control study. J Natl Cancer Inst 90:43–49.

Nguyen HH, Broker TR, Chow LT, Alvarez RD, Vu HL, Andrasi J, Brewer LR, Jin G and Mestecky J (2005): Immune responses to human papillomavirus in genital tract of women with cervical cancer. Gynecol Oncol 96:452–461.

Nieminen P, Vuorma S, Viikki M, Hakama, M and Anttila A (2004): Comparison of HPV test versus conventional and automation-assisted Pap screening as potential screening tools for preventing cervical cancer. BJOG 111:842–848.

Niu SP, Yang GH, Chen ZM, Wang JL, Wand GH, He XZ, Schoepff, Boreham J, Pan HC and Peto R (1998): Emerging tobacco hazards in China: 2. Early mortality results from a prospective study. BMJ 317:1423–1424.

Nygård JF, Skare GB and Thoresen SØ (2002): The cervical cancer screening programme in Norway, 1992-2000: changes in Pap smear coverage and incidence of cervical cancer. J Med Screen 9:86–91.

Olsen AO, Dillner J, Gjoen K and Magnus P (1997): Seropositivity against HPV16 capsids: a better marker of past sexual behaviour than presence of HPV DNA. Genitourinary Medicine 73:131–135.

Olsen AO, Orstavik I, Dillner J, Vestergaard BF and Magnus P (1998): Herpes simplex virus and human papillomavirus in a population-based case-control study of cervical intraepithelial neoplasia grade II-III. APMIS 106: 417–424.

Olsson SE, Villa LL, Costa RL, Petta CA, Andrade RP, Malm C, Iversen OE, Høye J, Steinwall M, Riis-Johannessen G, Andersson-Ellstrom A, Elfgren K, von Krogh G, Lehtinen M, Paavonen J, Tamms GM, Giacoletti K, Lupinacci L, Esser MT, Vuocolo SC, Saah AJ and Barr E (2007): Induction of immune memory following administration of a prophylactic quadrivalent human papillomavirus (HPV) types 6/11/16/18 L1 virus-like particle (VLP) vaccine. Vaccine 25:4931–4939.

Onda T, Carter JJ, Koutsky LA, Hughes JP, Lee S, Kuypers J, Kiviat N and Galloway DA (2003): Characterization of IgA response among women with incident HPV 16 infection. Virology 312:213–221.

Ouyang Y, Virasch N, Hao P, Aubrey MT, Mukerjee N, Bierer BE and Freed BM (2000): Suppression of human IL-1beta, IL-2, IFN-gamma, and TNF-alpha production by cigarette smoke extracts. J Allergy Clin Immunol 106:280–287.

Owen L and McNeill A (2001): Saliva cotinine as indicator of cigarette smoking in pregnant women. Addiction 96:1001–1006.

Paavonen J, Jenkins D, Bosch FX, Naud P, Salmerón J, Wheeler CM, Chow SN, Apter DL, Kitchener HC, Castellsague X, de Carvalho NS, Skinner SR, Harper DM, Hedrick JA, Jaisamrarn U, Limson GA, Dionne M, Quint W, Spiessens B, Peeters P, Struyf F, Wieting SL, Lehtinen MO and Dubin G (2007): HPV PATRICIA study group. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. Lancet 369:2161–2170.

Paavonen J, Naud P, Salmerón J, Wheeler CM, Chow SN, Apter D, Kitchener H, Castellsague X, Teixeira JC, Skinner SR, Hedrick J, Jaisamrarn U, Limson G, Garland S, Szarewski A, Romanowski B, Aoki FY, Schwarz TF, Poppe WA, Bosch FX, Jenkins D, Hardt K, Zahaf T, Descamps D, Struyf F, Lehtinen M, Dubin G; HPV PATRICIA Study Group, Greenacre M (2009): Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet 374: 301–314.

Pagliusi SR and Garland SM (2007): International standard reagents for HPV detection. Dis Markers 23:283–296.

Palefsky JM, Minkoff H, Kalish LA, Levine A, Sacks HS, Garcia P, Young M, Melnick S, Miotti P and Burk R(1999): Cervicovaginal human papillomavirus infection in human immunodeficiency virus-1 (HIV)-positive and high-risk HIV-negative women. J Natl Cancer Inst 91:226–236.

Parazzini F, Chatenoud L, La Vecchia C, Negri E, Franceschi S and Bolis G (1998): Determinants of risk of invasive cervical cancer in young women. Br J Cancer 77:838–841.

Parish S, Collins R, Peto R, Youngman L, Barton J, Jayne K, Clarke R, Appleby P, Lyon V and Cederholm-Williams S (1999): Cigarette smoking, tar yields, and non-fatal myocardial infarction: 14 000 cases and 32 000 controls in the United Kingdom. BMJ 311:471–477.

Park JS, Kim EJ, Kwon HJ, Hwang ES, Namkoong SE and Um SJ (2000): Inactivation of interferon regulatory factor-1 tumor suppressor protein by HPV E7 oncoprotein. Implication for the E7-mediated immune evasion mechanism in cervical carcinogenesis. J Biol Chem 275:6764–6769.

Parkin DM, Bray F, Ferlay J and Pisani P (2005): Global cancer statistics, 2002. CA Cancer J Clin 55:74–108.

Parkin DM, Sitas F, Chirenje M, Stein L, Abratt R and Wabinga H (2008): Part I: Cancer in Indigenous Africans--burden, distribution, and trends. Lancet Oncol 9:683–692.

Pastrana DV, Buck CB, Pang YY, Thompson CD, Castle PE, FitzGerald PC, Krüger Kjaer S, Lowy DR and Schiller JT (2004): Reactivity of human sera in a sensitive, high-throughput pseudovirus-based papillomavirus neutralization assay for HPV16 and HPV18. Virology 321:205–216.

Patel S and Chiplunkar S (2009): Host immune responses to cervical cancer. Curr Opin Obstet Gynecol 21:54–59.

Pedersen C, Petaja T, Strauss G, Rumke HC, Poder A, Richardus JH, Spiessens B, Descamps D, Hardt K, Lehtinen M and Dubin G (2007): HPV Vaccine Adolescent Study Investigators Network. Immunization of early adolescent females with human papillomavirus type 16 and 18 L1 virus-like particle vaccine containing AS04 adjuvant. J Adolesc Health 40:564–571.

Pérez-Stable EJ, Benowitz NL and Marín G (1995): Is serum cotinine a better measure of cigarette smoking than self-report? Preventive Medicine 24:171–179.

Peto R, Lopez AD, Boreham J, Thun M, Heath C Jr and Doll R (1996): Mortality from smoking worldwide. Br Med Bull 52:12–21.

Petäjä T, Keränen H, Karppa T, Kawa A, Lantela S, Siitari-Mattila M, Levänen H, Tocklin T, Godeaux O, Lehtinen M, Dubin G (2009). Immunogenicity and safety of human papillomavirus (HPV)-

16/18 AS04-adjuvanted vaccine in healthy boys aged 10-18 years. J Adolesc Health 44: 33–40.

Phillips DH and Shé MN (1994): DNA adducts in cervical tissue of smokers and non-smokers. Mutat Res 313:277–284.

Plummer M, Herrero R, Franceschi S, Meijer CJ, Snijders P, Bosch FX, de Sanjosé S and Muñoz N; IARC Multi-centre Cervical Cancer Study Group (2003): Smoking and cervical cancer: pooled analysis of the IARC multi-centric case-control study. Cancer Causes Control 14:805–814.

Poppe WA, Peeters R, Daenens P, Ide PS and Van Assche FA (1995): Tobacco smoking and the uterine cervix: cotinine in blood, urine and cervical fluid. Gynecol Obstet Invest 39:110–114.

Potischman N and Brinton LA(1996): Nutrition and cervical neoplasia. Cancer Causes Control 7:113–126. PRB World Population Data Sheet. Population Reference Bureau (PRB) http: //www. prb.org/ 2008.

Prokopczyk B, Trushin N, Leszczynska J, Waggoner SE and El-Bayoumy K (1999): Human cervical tissue metabolizes the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, via alpha-hydroxylation and carbonyl reduction pathways. Carcinogenesis 22:107–114.

Pukkala E, Andersen A, Berglund G, Gislefoss R, Gudnason V, Hallmans G, Jellum E, Jousilahti P, Knekt P, Koskela P, Kyyrönen PP, Lenner P, Luostarinen T, Löve A, Ogmundsdóttir H, Stattin P, Tenkanen L, Tryggvadóttir L, Virtamo J, Wadell G, Widell A, Lehtinen M and Dillner J (2007): Nordic biological specimen banks as basis for studies of cancer causes and control--more than 2 million sample donors, 25 million person years and 100,000 prospective cancers. Acta Oncologica 46:286–307.

Puranen MH, Yliskoski MH, Saarikoski SV, Syrjänen KJ and Syrjänen SM (1997): Exposure of an infant to cervical human papillomavirus infection of the mother is common. Am J Obstet Gynecol 176:1039–1045.

Puska P, Korhonen HJ, Uutela A, Helakorpi S and PihaT (1997): Anti-Smoking Policy in Finland. *In* "Smokefree Europe: A Forum for Networks" (P. Puska, L. Elovainio and H. Vertio, eds.), pp. 26–42. Finnish Centre for Health Promotion, Helsinki, Finland.

Rajkumar T, Franceschi S, Vaccarella S, Gajalakshmi V, Sharmila A, Snijders PJ, Muñoz N, Meijer CJ and Herrero R (2003): Role of paan chewing and dietary habits in cervical carcinoma in Chennai, India. Br J Cancer 88:1388–1393.

Reisinger KS, Block SL, Lazcano-Ponce E, Samakoses R, Esser MT, Erick J, Puchalski D, Giacoletti KE, Sings HL, Lukac S, Alvarez FB and Barr E (2007): Safety and persistent immunogenicity of a quadrivalent human papillomavirus types 6, 11, 16, 18 L1 viruslike particle vaccine in preadolescents and adolescents: a randomized controlled trial. Pediatr Infect Dis J 26:201–209.

Ries LAG, Melbert D, Krapcho M, Stinchcomb DG, Howlader N, Horner MJ, Mariotto A, Miller BA, Feuer EJ, Altekruse SF, Lewis DR, Clegg L, Eisner MP, Reichman M and Edwards BK (2007): SEER Cancer Statistics Review, 1975–2005, National Cancer Institute. Bethesda, MD. Based on November 2007 SEER data submission, posted to the SEER web site http: //seer.cancer. gov/csr/1975_ 2005/, 2008.

Rimpelä AH and Rainio SU (2004): The effectiveness of tobacco sales ban to minors: the case of Finland. Tob Control 13:167–174.

Rintala MA, Grénman SE, Puranen MH, Isolauri E, Ekblad U, Kero PO, Syrjänen SM (2005). Transmission of high-risk human papillomavirus (HPV) between parents and infant: a prospective study of HPV in families in Finland. J Clin Microbiol 43: 376–381.

Roberts S, Ashmole I, Gibson LJ, Rookes SM, Barton GJ and Gallimore PH (1994): Mutational analysis of human papillomavirus E4 proteins: identification of structural features important in the formation of cytoplasmic E4/cytokeratin networks in epithelial cells. J Virol 68:6432–6445.

Roden R and Wu TC (2006): How will HPV vaccines affect cervical cancer? Nat Rev Cancer 6:753–763.

Rolón PA, Smith JS, Muñoz N, Klug SJ, Herrero R, Bosch X, Llamosas F, Meijer CJ and Walboomers JM (2000): Human papillomavirus infection and invasive cervical cancer in Paraguay. Int J Cancer 85:486–491.

Ronco G, Cuzick J, Pierotti P, Cariaggi MP, Dalla Palma P, Naldoni C, Ghiringhello B, Giorgi-Rossi P, Minucci D, Parisio F, Pojer A, Schiboni ML, Sintoni C,

Zorzi M, Segnan N and Confortini M (2007): Accuracy of liquid based versus conventional cytology: overall results of new technologies for cervical cancer screening: randomised controlled trial. BMJ 335:28.

Rosales R, López-Contreras M and Cortes RR (2001): Antibodies against human papillomavirus (HPV) type 16 and 18 E2, E6 and E7 proteins in sera: correlation with presence of papillomavirus DNA. J Med Virol 65:736–744.

Rousseau MC, Abrahamowicz M, Villa LL, Costa MC, Rohan TE and Franco EL (2003): Predictors of cervical coinfection with multiple human papillomavirus types. Cancer Epidemiol Biomarkers Prev 12:1029–1037.

Rowhani-Rahbar A, Hawes SE, Sow PS, Toure P, Feng Q, Dem A, Dembele B, Critchlow CW, N'Doye I and Kiviat NB (2007): The impact of HIV status and type on the clearance of human papillomavirus infection among Senegalese women. J Infect Dis 196:887–894.

Sadjadi A, Malekzadeh R, Derakhshan MH, Sepehr A, Nouraie M, Sotoudeh M, Yazdanbod A, Shokoohi B, Mashayekhi A, Arshi S, Majidpour A, Babaei M, Mosavi A, Mohagheghi MA and Alimohammadian M (2003): Cancer occurrence in Ardabil: results of a population-based cancer registry from Iran. Int J Cancer 107:113–118.

Sankaranarayanan R, Gaffikin L, Jacob M, Sellors J and Robles S (2005): A critical assessment of screening methods for cervical neoplasia. Int J Gynaecol Obstet 89: S4–S12.

Sankaranarayanan R, Nene BM, Shastri SS, Jayant K, Muwonge R, Budukh AM, Hingmire S, Malvi SG, Thorat R, Kothari A, Chinoy R, Kelkar R, Kane S, Desai S, Keskar VR, Rajeshwarkar R, Panse N, Dinshaw KA (2009): HPV screening for cervical cancer in rural India. N Engl J Med 360: 1385–1394.

Santos C, Muñoz N, Klug S, Almonte M, Guerrero I, Alvarez M, Velarde C, Galdos O, Castillo M, Walboomers J, Meijer C and Caceres E (2001): HPV types and cofactors causing cervical cancer in Peru. Br J Cancer 85:966–971.

Sarian LO, Derchain SF, Naud P, Roteli-Martins C, Longatto-Filho A, Tatti S, Branca M, Erzen M, Serpa-Hammes L, Matos J, Gontijo R, Bragança JF, Lima TP, Maeda MY, Lörincz A, Dores GB, Costa S, Syrjänen S and Syrjänen K (2005): Evaluation of visual inspection with acetic acid (VIA), Lugol's iodine (VILI), cervical cytology and HPV testing as cervical screening tools in Latin America. This report refers to partial results from the LAMS (Latin AMerican Screening) study. J Med Screen 12:142–149.

Sasagawa T, Inoue M, Lehtinen M, Zhang W, Gschmeissner SE, Hajibagheri MA, Finch J and Crawford L (1996): Serological responses to human papillomavirus type 6 and 16 virus-like particles in patients with cervical neoplastic lesions. Clin Diagn Lab Immunol 3:403–410.

Scherer G and Richter E (1997): Biomonitoring exposure to environmental tobacco smoke (ETS): A critical reappraisal. Human and Experimental Toxicology 16:449–459.

Schiffman M, Hildesheim A, Herrero R and Bratti C (2000): Human papillomavirus testing as a screening tool for cervical cancer. JAMA 283:2525–2526.

Schiller JT and Hidesheim A (2000): Developing HPV virus-like particles vaccines to prevent cervical cancer: a progress report. J Clin Virol 19:74–76.

Schlecht NF, Platt RW, Duarte-Franco E, Costa MC, Sobrinho JP, Prado JC, Ferenczy A, Rohan TE, Villa LL and Franco EL (2003): Human papillomavirus infection and time to progression and regression of cervical intraepithelial neoplasia. J Natl Cancer Inst 95:1336–1343.

Schmitt M, Bravo IG, Snijders PJ, Gissmann L, Pawlita M and Waterboer T (2006): Bead-based multiplex genotyping of human papillomaviruses. J Clin Microbiol 44: 504–512.

Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) (2007): Health Effects of Smokeless Tobacco Products. Preliminary Report. European Union. http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_009.pdf. Acces online 10 March 2009.

Secker-Walker RH, Vacek PM, Flynn BS and Mead PB (1997): Exhaled carbon monoxide and urinary cotinine as measures of smoking in pregnancy. Addict Behav 22: 671–684.

Sellors JW and Sankaranarayanan R (2003): Colposcopy and treatment on cervical intraepithelial neoplasia: A beginners' manual. Lyon: IARCPress.

Seo YS, Müller F, Lusky M, Gibbs E, Kim HY, Phillips B and Hurwitz J (1993): Bovine papilloma virus (BPV)-encoded E2 protein enhances binding of E1 protein to the BPV replication origin. Proc Natl Acad Sci U S A 90:2865–2869.

Shafey O, Dolwick S and Guindon GE, eds (2004): Tobacco Control Country Profiles 2003. American Cancer Society, Inc, Atlanta.

Sherman ME, Lorincz AT, Scott DR, Wacholder S, Castle PE, Glass AG, Mielzynska-Lohnas I, Rush BB and Schiffman M (2003): Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. J Natl Cancer Inst 95:46–52.

Silins I, Avall-Lundqvist E, Tadesse A, Jansen KU, Stendahl U, Lenner P, Zumbach K, Pawlita M, Dillner J and Frankendal B (2002): Evaluation of antibodies to human papillomavirus as prognostic markers in cervical cancer patients. Gynecol Oncol 85:333–338.

Silins I, Kallings I and Dillner J (2000): Correlates of the spread of human papillomavirus infection. Cancer Epidemiol Biomarkers Prev 9:953–959.

Silins I, Ryd W, Strand A, Wadell G, Tornberg S, Hansson BG, Wang X, Arnheim L, Dahl V, Bremell D, Persson K, Dillner J and Rylander E (2005): Chlamydia trachomatis infection and persistence of human papillomavirus. Int J Cancer 116:110–115.

Simonetti AC, Melo JH, de Souza PR, Bruneska D and de Lima Filho JL (2009): Immunological's host profile for HPV and Chlamydia trachomatis, a cervical cancer cofactor. Microbes Infect 11:435–442

Simons AM, Mugica Van Heckenrode C, Rodriguez JA, Maitland N, Anderson M, Phillips DH and Coleman DV (1995): Demonstration of smoking-related DNA damage in cervical epithelium and correlation with human papillomavirus type 16, using exfoliated cervical cells. Br J Cancer 71:246–249.

Sinclair KA, Woods CR, Kirse DJ and Sinal SH (2005): Anogenital and respiratory tract human papillomavirus infections among children: age, gender, and potential transmission through sexual abuse. Pediatrics 116:815–825.

Six C, Heard I, Bergeron C, Orth G, Poveda JD, Zagury P, Cesbron P, Crenn-Hebert C, Pradinaud R, Sobesky M, Marty C, Babut ML, Malkin JE, Odier A, Fridmann S, Aubert JP and Brunet JB (1998): Comparative prevalence, incidence and short-term prognosis of cervical squamous intraepithelial lesions amongst HIV-positive and HIV-negative women. AIDS 12:1047–1056.

Sjöholm MI, Dillner J and Carlson J (2007): Assessing quality and functionality of DNA from fresh and archival dried blood spots and recommendations for quality control guidelines. Clin Chem 53:1401–1407.

Smith JS, Green J, Berrington de GA, Appleby P, Peto J, Plummer M, Franceschi S and Beral V (2003): Cervical cancer and use of hormonal contraceptives: a systematic review. Lancet 361:1159–1167.

Smith JS, Herrero R, Bosetti C, Munoz N, Bosch FX, Eluf-Neto J, Castellsague X, Meijer CJ, Van den Brule AJ, Franceschi S and Ashley R (2002): Herpes simplex virus-2 as a human papillomavirus cofactor in the etiology of invasive cervical cancer. J Natl Cancer Inst 94:1604–1613.

Snijders PJ, Hogewoning CJ, Hesselink AT, Berkhof J, Voorhorst FJ, Bleeker MC and Meijer CJ (2006): Determination of viral load thresholds in cervical scrapings to rule out CIN 3 in HPV 16, 18, 31 and 33-positive women with normal cytology. Int J Cancer 119:1102–1107.

Snijders PJ, van den Brule AJ and Meijer CJ (2003): The clinical relevance of human papillomavirus testing: relationship between analytical and clinical sensitivity. J Pathol 201:1–6.

SNRT Subcommittee on Biochemical Verification (2002). Nicotine & Tobacco Research 4:149–159.

Solomon R (2001): New procedure under study may replace Pap smear. Posit Living 10:34.

Soost HJ, Lange HJ, Lehmacher W and Ruffing-Kullmann B (1991): The validation of cervical cytology. Sensitivity, specificity and predictive values. Acta Cytol 35: 8–14.

Stanfill SB, Calafat AM, Brown CR, Polzin GM, Chiang JM, Watson CH and Ashley DL (2003): Concentrations of nine alkenylbenzenes, coumarin, piperonal and pulegone in Indian bidi cigarette tobacco. Food chem Toxicol 41:303–317.

Stanley M, Lowy DR and Frazer I (2006): Chapter 12: Prophylactic HPV vaccines: underlying mechanisms. Vaccine 24 Suppl 3:S3/106–113.

Stanley M (2008): Immunobiology of HPV and HPV vaccines. Gynecol Oncol 109:S15–S21.

Stanley MA, Pett MR and Coleman N (2007): HPV: from infection to cancer. Biochem Soc Trans 35:1456–1460.

Stellman SD, Austin H and Wynder EL (1980): Cervix cancer and cigarette smoking: a case-control study. Am J Epidemiol 111:383–388.

Stewart BW and Kleihues P (2003): World Cancer Report. International Agency for Research on Cancer. IARCPress, Lyon, France.

Stewart DE, Gagliardi A, Johnston M, Howlett R, Barata P, Lewis N, Oliver T and Mai V (2007): Self-collected samples for testing of oncogenic human papillomavirus: a systematic review. J Obstet Gynaecol Can 29: 817–828.

Stewart RD (1975): The effect of carbon monoxide on humans. Annual Review of Pharmacology 15:409–423.

Stevens-Simon C, Nelligan D, Breese P, Jenny C and Douglas JM Jr (2000): The prevalence of genital human papillomavirus infections in abused and nonabused preadolescent girls. Pediatrics 106:645–649.

Stratton K, Shetty P, Wallace R and Bonduras S, eds (2001): Products for tobacco exposure reduction. In: *Clearing the smoke. Assessing the science based for tobacco harm reduction*, Washington DC, National Academy Press pp. 82–92.

Strickler HD, Palefsky JM, Shah KV, Anastos K, Klein RS, Minkoff H, Duerr A, Massad LS, Celentano DD, Hall C, Fazzari M, Cu-Uvin S, Bacon M, Schuman P, Levine AM, Durante AJ, Gange S, Melnick S and Burk RD (2003): Human papillomavirus type 16 and immune status in human immunodeficiency virusseropositive women. J Natl Cancer Inst 95:1062–1071.

Stubenrauch F and Laimins LA (1999): Human papillomavirus life cycle: active and latent phases. Semin Cancer Biol 9:379–386.

Subramanya D and Grivas PD (2008): HPV and cervical cancer: updates on an established relationship. Postgrad Med 120:1–7.

Suk K, Chang I and Kim YH (2001): Interferon γ (IFNγ) and tumor necrosis factor α synergism in ME-180 cervical cancer apoptosis and necrosis. IFNγ inhibits cytoprotective NK-$_K$B through STAT1/ IRF-1 pathways. J Biol Chem 276:153–159.

Sun Y, Eluf-Neto J, Bosch FX, Muñoz N, Booth M, Walboomers JM, Shah KV and Viscidi RP (1994): Human papillomavirus-related serological markers of invasive cervical carcinoma in Brazil. Cancer Epidemiol Biomarkers Prev 3:341–347.

Suprynowicz FA, Disbrow GL, Krawczyk E, Simic V, Lantzky K and Schlegel R (2008): HPV-16 E5 oncoprotein upregulates lipid raft components caveolin-1 and ganglioside GM1 at the plasma membrane of cervical cells. Oncogene 27:1071–1078.

Svare EI, Kjaer SK, Worm AM, Osterlind A, Moi H, Christensen RB, Meijer CJ, Walboomers JM and van den Brule AJ (1998): Risk factors for HPV infection in women from sexually transmitted disease clinics: comparison between two areas with different cervical cancer incidence. Int J Cancer 75:1–8.

Sverdrup F and Khan SA (1995): Two E2 binding sites alone are sufficient to function as the minimal origin of replication of human papillomavirus type 18 DNA. J Virol 69:1319–1323.

Syrjänen K, Shabalova I, Petrovichev N, Kozachenko V, Zakharova T and Pajanidi J (2007): Smoking is an independent risk factor for oncogenic human papillomavirus (HPV) infections but not for high-grade CIN. Eur J Epidemiol 22:723–735.

Syrjänen S and Syrjänen K (1986):. An improved in situ hybridization protocol for detection of human papillomavirus (HPV) DNA sequences in paraffin-embedded biopsies. J Virol Meth 14:293–304.

Szarewski A, Jarvis JJ, Sasieni P, Anderson M, Edwards R, Steele SJ, Guillebaud J and Cuzick J (1996): Effect of smoking cessation on cervical lesion size. Lancet 347:941–943.

Szarewski A, Maddox P, Royston P, Jarvis M, Anderson M and Guillebaud J (2001): The effects of stopping smoking on cervical Langerhans'cells and lymphocytes. Br J Obstet Gynecol 108:295–303.

Tedeschi R, Bidoli E, Agren A, Hallmans G, Wadell G, De Paoli P and Dillner J (2006): Epidemiology of Kaposi's Sarcoma Herpesvirus (HHV8) in Västerbotten County, Sweden. Journal of Medical Virology 78:372–378.

Terry G, Ho L, Londesborough P, Cuzick J, Mielzynska-Lohnas I and Lorincz A (2001): Detection of high-risk HPV types by the hybrid capture 2 test. J Med Virol 65:155–162.

The Future II study Group (2007): Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl J Med 356:1915–1927.

Thomas JO, Herrero R, Omigbodun AA, Ojemakinde K, Ajayi IO, Fawole A, Oladepo O, Smith JS, Arslan A, Muñoz N, Snijders PJ, Meijer CJ and Franceschi S (2004): Prevalence of papillomavirus infection in women in Ibadan, Nigeria: a population-based study. Br J Cancer 90:638–645.

Tindle RW (2002): Immune evasion in human papillomavirus-associated cervical cancer. Nat Rev Cancer 2:59–65.

Tolstrup J, Munk C, Lykke Thomsen B, Svare E, Van Den Brule AJC, Grønæk M Meijer C and Kjaer Krüger S (2006): The role of smoking and alcohol intake in the development of high-grade squamous intraepithelial lesions among high – risk HPV-positive women. Acta Obstet Gynecol Scand 85:1114–1119.

Torell U (2002): The smoking man. [Den rökande människan] Dissertation. Linköping University. Linköping.

Tso TC (1991): The production of tobacco. In: Production, physiology and biochemistry of tobacco plant, Beltsville, MD, Ideals, pp 55–64.

Tulinius H, Sigfússon N, Sigvaldason H, Bjarnadóttir K and Tryggvadóttir L (1997): Risk factors for malignant diseases: a cohort study on a population of 22,946 Icelanders. Cancer Epidemiol Biomarkers Prev 6:863–873.

Tverdal A, Thelle D, Stensvold I, Leren P and Bjartveit K (1993): Mortality in relation to smoking history: 13 years' follow-up of 68,000 Norwegian men and women 35-49 years. J Clin Epidemiol 46:475–487.

Um SJ, Lee SY, Kim EJ, Myoung J, Namkoong SE and Park JS (2002): Down-regulation of human papillomavirus E6/E7 oncogene by arsenic trioxide in cervical carcinoma cells. Cancer Lett 181:11–22.

Ursin G, Pike MC, Preston-Martin S, d'Ablaing G 3rd and Peters RK (1996): Sexual, reproductive, and other risk factors for adenocarcinoma of the cervix: results from a population-based case-control study (California, United States). Cancer Causes Control 7:391–401.

Wagenknecht LE, Cutter GR, Haley NJ, Sidney S, Manolio TA and Hughes GH (1990): Racial differences in serum cotinine levels among smokers in the Coronary Artery Risk Development in (Young) Adults study. Am J Public Health 80:1053–1056.

Wahi RN (1998): The Epidemiology of oral and oropharyngeal cancer. A report of the study in Mainpuri district, Uttar Pradesh, India. Bulletin of the World Health Organization 38:495–521.

Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ and Munoz N (1999): Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 189:12–19.

Valle GF and Banks L (1995): The human papillomavirus (HPV)-6 and HPV-16 E5 proteins co-operate with HPV-16 E7 in the transformation of primary rodent cells. J Gen Virol 76:1239–1245.

Wallin KL, Wiklund F, Luostarinen T, Angstrom T, Anttila T, Bergman F, Hallmans G, Ikaheimo I, Koskela P, Lehtinen M, Stendahl U, Paavonen J and Dillner J (2002): A population-based prospective study of Chlamydia trachomatis infection and cervical carcinoma. Int J Cancer 101:371–374.

van den Brule AJ, Pol R and Fransen-Daalmeijer N (2002): GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes, J Clin Microbiol 40:779–787.

van der Burg SH, Piersma SJ, de Jong A, van der Hulst JM, Kwappenberg KM, van den Hende M, Welters MJ, Van Rood JJ, Fleuren GJ, Melief CJ, Kenter GG and Offringa R (2007): Association of cervical cancer with the presence of CD4+ regulatory T cells specific for human papillomavirus antigens. Proc Natl Acad Sci U S A 104:12087–12092.

Van der Eb MM, Leyten EM, Gavarasana S, Vandenbroucke JP, Kahn PM and Cleton FJ (1993): Reverse smoking as a risk factor for palatal cancer: a cross-sectional study in rural Andhra Pradesh, India. Int J Cancer 54:754–758.

van Duin M, Snijders PJ, Schrijnemakers HF, Voorhorst FJ, Rozendaal L, Nobbenhuis MA, van den Brule AJ, Verheijen RH, Helmerhorst TJ and Meijer CJ (2002):

Human papillomavirus 16 load in normal and abnormal cervical scrapes: an indicator of CIN II/III and viral clearance. Int J Cancer 98:590–595.

Wang KL (2007): Human papillomavirus and vaccination in cervical cancer. Taiwan J Obstet Gynecol 46:352–362.

Wang SS and Hildesheim A (2003): Chapter 5: Viral and host factors in human papillomavirus persistence and progression. J Natl Cancer Inst Monogr 31:35–40.

Wang ZH, Kjellberg L, Abdalla H, Wiklund F, Eklund C, Knekt P, Lehtinen M, Kallings I, Lenner P, Hallmans G, Mählck CG, Wadell G, Schiller J and Dillner J (2000): Type specificity and significance of different isotypes of serum antibodies to human papillomavirus capsids. J Infect Dis 181:456–462.

Vartiainen E, Seppälä T, Lillsunde P and Puska P (2002): Validation of self reported smoking by serum cotinine measurement in a community-based study. J Epidemiol Community Health 56:167–170.

Velicer WF, Prochaska JO, Rossi JS and Snow MG (1992): Assessing outcome in smoking cessation studies. Psychological Bulletin 111:23–41.

Wheeler CM (2008): Natural history of human papillomavirus infections, cytologic and histologic abnormalities, and cancer. Obstet Gynecol Clin North Am 35:519–536.

Whetzel CA, Corwin EJ and Klein LC (2007): Disruption in Th1/Th2 immune response in young adult smokers. Addict Behav 32:1–8.

Whiteside MA, Siegel EM and Unger ER (2008): Human papillomavirus and molecular considerations for cancer risk. Cancer 113:2981–2994.

Wideroff L, Schiffman M, Haderer P, Armstrong A, Greer CE, Manos MM, Burk RD, Scott DR, Sherman ME, Schiller JT, Hoover RN, Tarone RE and Kirnbauer R (1999): Seroreactivity to human papillomavirus types 16, 18, 31, and 45 virus-like particles in a case-control study of cervical squamous intraepithelial lesions. J Infect Dis 180:1424–1428.

Wiley DJ, Wiesmeier E, Masongsong E, Gylys K, Koutsky L, Ferris DG and the Proof of Principle Study Investigative Group (2006): Smokers at higher risk for undetected antibody for oncogenic human papillomavirus type 16 infection. Cancer Epidemiol Biomarkers Prev 15:915–920.

Villa LL, Ault KA, Giuliano AR, Costa RL, Petta CA, Andrade RP, Brown DR, Ferenczy A, Harper DM, Koutsky LA, Kurman RJ, Lehtinen M, Malm C, Olsson SE, Ronnett BM, Skjeldestad FE, Steinwall M, Stoler MH, Wheeler CM, Taddeo FJ, Yu J, Lupinacci L, Railkar R, Marchese R, Esser MT, Bryan J, Jansen KU, Sings HL, Tamms GM, Saah AJ and Barr E (2006): Immunologic responses following administration of a vaccine targeting human papillomavirus Types 6, 11, 16, and 18. Vaccine 24:5571–5583.

Villa LL, Costa RL, Petta CA, Andrade RP, Ault KA, Giuliano AR, Wheeler CM, Koutsky LA, Malm C, Lehtinen M, Skjeldestad FE, Olsson SE, Steinwall M, Brown DR, Kurman RJ, Ronnett BM, Stoler MH, Ferenczy A, Harper DM, Tamms GM, Yu J, Lupinacci L, Railkar R, Taddeo FJ, Jansen KU, Esser MT, Sings HL, Saah AJ and Barr E (2005): Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebocontrolled multicentre phase II efficacy trial. Lancet Oncol 6:271–278.

Wills TA and Cleary SD (1997): The validity of self-reports of smoking: Analyses by race/ethnicity in a school sample of urban adolescents. Am J Public Health 87:56–61.

Wilson VG, West M, Woytek K and Rangasamy D (2002): Papillomavirus E1 proteins: form, function, and features. Virus Genes 24:275–290.

Winkelstein JW (1977): Smoking and cervical cancer of the uterine cervix: hypothesis. Am J Epidemiol 106:257–259.

Wolfers J, Lozier A, Raposo G, Regnault A, Thery C, Masurier C, Flament C, Pouzieux S, Faure F, Tursz T, Angevin E, Amigorena S and Zitvogel L (2001): Tumorderived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. Nat Med 7:297–303

Volpers C, Unckell F, Schirmacher P, Streeck RE and Sapp M (1995): Binding and internalization of human papillomavirus type 33 virus-like particles by eukaryotic cells. J Virol 69:3258–3264.

Woodhouse SL, Stastny JF, Styer PE, Kennedy M, Praestgaard AH and Davey DD (1999): Interobserver variability

in subclassification of squamous intraepithelial lesions: Results of the College of American Pathologists Interlaboratory Comparison Program in Cervicovaginal Cytology. Arch Pathol Lab Med 123: 1079–1084.

Woodman CB, Collins SI and Young LS (2007): The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer 7:11–22.

Woodward M, Tunstall-Pedoe H, Smith WC and Tavendale R (1991): Smoking characteristics and inhalation biochemistry in the Scottish population. Journal of Clinical Epidemiology 44:1405–1410.

World Bank (1999): Curbing the epidemic: governments and the economics of tobacco control. The World Bank. Tob Control 8:196–201.

World Health Organisation (2008): WHO Report on the Global Tobacco Epidemic, 2008 - The MPOWER package. http://www.who.int/tobacco/mpower/gtcr_download/en/index.html.

World Health Organisation (2003): World cancer report. IARC Press, Lyon, France.

Xi LF, Carter JJ, Galloway DA, Kuypers J, Hughes JP, Lee SK, Adam DE, Kiviat NB and Koutsky LA (2002): Acquisition and natural history of human papillomavirus type 16 variant infection among a cohort of female university students. Cancer Epidemiol Biomarkers Prev11:343–351.

Yang X, Jin G, Nakao Y, Rahimtula M, Pater MM and Pater A (1996): Malignant transformation of HPV 16-immortalized human endocervical cells by cigarette smoke condensate and characterization of multistage carcinogenesis. Int J Cancer 65:338–344.

Ylitalo N, Sørensen P, Josefsson AM, Magnusson PK, Andersen PK, Pontén J, Adami HO, Gyllensten UB and Melbye M (2000): Consistent high viral load of human papillomavirus 16 and risk of cervical carcinoma in situ: a nested case-control study. Lancet 355:2194–2198.

Yoshikawa H, Nagata C, Noda K, Nozawa S, Yajima A, Sekiya S, Sugimori H, Hirai Y, Kanazawa K, Sugase M, Shimizu H and Kawana T (1999): Human papillomavirus infection and other risk factors for cervical intraepithelial neoplasia in Japan. Br J Cancer 80:621–624.

Zehbe I, Höhn H, Pilch H, Neukirch C, Freitag K and Maeurer MJ (2005): Differential MHC class II component expression in HPV-positive cervical cancer cells: implication for immune surveillance. Int J Cancer 117:807–815.

Zelmanowicz AM, Schiffman M, Herrero R, Goldstein AM, Sherman ME, Burk RD, Gravitt P, Viscidi R, Schwartz P, Barnes W, Mortel R, Silverberg SG, Buckland J and Hildesheim A (2005): Family history as a co-factor for adenocarcinoma and squamous cell carcinoma of the uterine cervix: results from two studies conducted in Costa Rica and the United States. Int J Cancer 116:599–605.

Zhou J, Liu WJ, Peng SW, Sun XY and Frazer I (1999): Papillomavirus capsid protein expression level depends on the match between codon usage and tRNA availability. J Virol 73:4972–4982.

Zondervan KT, Carpenter LM, Painter R and Vessey MP (1996): Oral contraceptives and cervical cancer--further findings from the Oxford Family Planning Association contraceptive study. Br J Cancer 73:1291–1297.

zur Hausen H (2002): Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2:342–350.

zur Hausen H (2006): Streptococcus bovis: causal or incidental involvement in cancer of the colon? Int J Cancer 119:xi–xii.

Zur Haussen H (1999): Viruses in human cancers. Eur J Cancer 35:1878–1885.