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Mass Screening Registry,
Finnish Cancer Registry

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Cancer incidence, mortality, and pregnancy outcome among women treated for cervical intraepithelial neoplasia.

Academic Dissertation

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To my family

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1 Abstract

Cervical cancer develops through precursor lesions, i.e. cervical intraepithelial neoplasms (CIN). These can be detected and treated before progression to invasive cancer. The major risk factor for developing cervical cancer or CIN is persistent or recurrent infection with high-risk human papilloma virus (hrHPV). Other associated risk factors include low socio-economic status, smoking, sexually transmitted infections, and high number of sexual partners, and these risk factors can predispose to some other cancers, excess mortality, and reproductive health complications as well.

The aim was to study long-term cancer incidence, mortality, and reproductive health outcomes among women treated for CIN. Based on the results, we could evaluate the efficacy and safety of CIN treatment practices and estimate the role of the risk factors of CIN patients for cancer incidence, mortality, and reproductive health.

We collected a cohort of 7 599 women treated for CIN at Helsinki University Central Hospital from 1974 to 2001. Information about their cancer incidence, cause of death, birth of children and other reproductive endpoints, and socio-economic status were gathered through register-linkages to the Finnish Cancer Registry, Finnish Population Registry, and Statistics Finland. Depending on the endpoints in question, the women treated were compared to the general population, to themselves, or to an age- and municipality-matched reference cohort.

Cervical cancer incidence was increased after treatment of CIN for at least 20 years, regardless of the grade of histology at treatment. Compared to all of the colposcopically guided methods, cold knife conization (CKC) was the least effective method of treatment in terms of later CIN 3 or cervical cancer incidence. In addition to cervical cancer, incidence of other HPV-related anogenital cancers was increased among those treated, as was the incidence of lung cancer and other smoking-related cancers.

Mortality from cervical cancer among the women treated was not statistically significantly elevated, and after adjustment for socio-economic status, the hazard ratio (HR) was 1.0. In fact, the excess mortality among those treated was mainly due to increased mortality from other cancers, especially from lung cancer.

In terms of post-treatment fertility, the CIN treatments seem to be safe: The women had more deliveries, and their incidence of pregnancy was similar before and after treatment. Incidence of extra-uterine pregnancies and induced abortions was elevated among the treated both before and after treatment. Thus this elevation did not occur because they were treated — rather to a great extent was due to the other known risk factors these women had in excess, i.e. sexually transmitted infections.

The purpose of any cancer preventive activity is to reduce cancer incidence and mortality. In Finland, cervical cancer is a rare disease and death from it even rarer, mostly due to the effective screening program. Despite this, the women treated are at increased risk for cancer; not just for cervical cancer. They must be followed up carefully and for a long period of time; general health education, especially cessation of smoking, is crucial in the management process, as well as interventions towards proper use of birth control such as condoms.

2 Finnish summary

Kohdunkaulan syöpä kehittyy todettavien ja hoidettavien esiasteiden kautta. Tunnetuin ja tärkein riskitekijä sekä kohdunkaulan syövän, että kohdunkaulan syövän esiasteiden kehittymiselle on suuren riskin ihmisen papilloomavirusinfektio (hrHPV). Muita merkittäviä riskitekijöitä ovat mm. sukupuoliteitse välittyvät taudit sekä tupakointi, jotka ovat riskitekijöitä myös tietyille muille syöville, lisääntyneelle kuolleisuudelle sekä lisääntymisterveyteen liittyville komplikaatioille.

Väitöskirjatutkimuksen tavoitteena oli selvittää pitkän aikavälin syöpäilmaantuvuutta, -kuolleisuutta sekä lisääntymisterveystapahtumia kohdunkaulan syövän esiasteesta hoidetuilla naisilla. Tulosten perusteella oli tarkoitus arvioida esiastehoitojen vaikuttavuutta ja turvallisuutta, sekä kartoittaa muiden tässä joukossa lisääntyneiden riskitekijöiden vaikutusta yleiseen sairastavuuteen ja kuolleisuuteen.

Tutkimusaineisto koostui 7599:stä HYKS Naistenklinikalla vv. 1974–2001 kohdunkaulan syövän esiasteesta hoidetusta naisesta. Tieto myöhemmästä syöpäilmaantuvuudesta, kuolinsyistä, lasten syntymäpäivistä, muista lisääntymisterveydellisistä muuttujista, sekä sosioekonomisen aseman luokitus hankittiin yhdistämällä tutkimusaineisto Suomen Syöpärekisterin, Väestörekisterin sekä STAKESin (nyk THL) kanssa. Riippuen osatutkimuksesta, hoidettuja naisia vertailtiin joko muuhun väestöön, itseensä, tai ikä- ja asuinkuntakaltaistettuun vertailuväestöön.

Kohdunkaulan syövän ilmaantuvuus oli koholla kahdenkymmen vuoden ajan hoidetun levyepiteeliperäisen esiasteen jälkeen, riippumatta esiasteen vaikeusasteesta. Myös muiden HPV-riippuvaisten anogenitaalialueen syöpien (vagina-, vulva- ja anussyöpä), keuhkosyövän ja muiden tupakointiin liittyvien syöpien ilmaantuvuus oli koholla esiastehoidetuilla naisilla muuhun väestöön verrattuna. Eri hoitomuotojen keskinäisessä vertailussa CIN 3- ja kohdunkaulan syövän ilmaantuvuus oli suurinta veitsikonisaation jälkeen.

Hoidon jälkeisen fertiliteetin suhteen esiastehoito on turvallinen: Esiasteesta hoidetut naiset tulivat vertailuväestöä useammin raskaaksi, sekä synnyttivät useammin hoidon jälkeen verrattuna hoitoa edeltäneeseen ajanjaksoon. Kohdunulkoisten raskauksien ja raskaudenkeskeytysten ilmaantuvuus oli hoidetuilla verrokkeja suurempaa sekä ennen että jälkeen hoidon: Kyseinen havainto ei siis liity itse hoitoon vaan pikemminkin muihin riskitekijöihin, mm. klamydiainfektioon, joita esiastepotilailla on keskimäärin muuta väestöä yleisemmin.

Minkä tahansa seulontaohjelman tai muun syövänehkäisytoiminnan lopullisena päämääränä on ehkäistä syöpäkuolleisuutta. Suomessa kohdunkaulan syöpä on nykyään harvinainen sairaus ja kuolema tähän syöpään on vieläkin harvinaisempaa, ennen kaikkea tehokkaan seulontaohjelman ansiosta. Tästä huolimatta sekä kohdunkaulan että muutamien muiden syöpien riski on kohdunkaulan syövän esiasteen sairastaneilla lisääntynyt muuhun väestöön verrattuna. Hoidettuja naisia pitää seurata tarkasti ja riittävän pitkän ajan. Yleinen terveysvalistus, erityisesti kannustaminen tupakoinnin lopettamiseen ja kondomin käyttöön ovat tässä toiminnassa erityisasemassa.

3 Original Publications

This thesis is based on the following original publications, which are referred to in the text by their roman numerals.

I Kalliala I, Anttila A, Pukkala E, Nieminen P. Risk of cervical and other cancers after treatment of Cervical intraepithelial neoplasia: a retrospective cohort study BMJ 2005; 331: 1183–1185

II Kalliala I, Nieminen P, Dyba T, Pukkala E, Anttila A. Cancer free survival after CIN treatment: comparisons of treatment methods and histology. Gynecologic Oncology 2007; 105: 228–233

III Kalliala I, Dyba T, Nieminen P, Hakulinen T, Anttila A. Mortality in a long-term follow-up after treatment of CIN. Int J Cancer. 2010; 126: 224–31.

IV Kalliala I, Anttila A, Dyba T, Hakulinen T, Halttunen M, Nieminen P. Fertility and pregnancy outcome among cervical intraepithelial neoplasia patients: a retrospective cohort study. Submitted

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4 Abbreviations

AIS	adenocarcinoma in situ
AGC-FN	atypical glandular cells, favor neoplasia
AGC-NOS	atypical glandular cells not otherwise specified
ASC-H	atypical squamous cells, HSIL not excluded
ASC-US	atypical squamous cells of unknown significance
CI	95% confidence interval
CIGN	cervical intraepithelial glandular neoplasia
CIGN 1	cervical intraepithelial glandular neoplasia, grade 1
CIGN 2	cervical intraepithelial glandular neoplasia, grade 2
CIN	cervical intraepithelial neoplasia
CIN 1	cervical intraepithelial neoplasia, grade 1
CIN 2	cervical intraepithelial neoplasia, grade 2
CIN 3	cervical intraepithelial neoplasia, grade 3
CIN 3+	cervical intraepithelial neoplasia, grade 3 or invasive carcinoma
CIN NOS	cervical intraepithelial neoplasia, grade not otherwise specified
CKC	cold knife conization
Cryo	cryocoagulation
DNA	deoxyribonucleic acid
HIV	human immunodeficiency virus
HPV	human papilloma virus

HR	hazard ratio
hrHPV	high-risk human papilloma virus
HSIL	high-grade squamous intraepithelial lesion
ICD	International Classification of Diseases
IRR	incidence relative risk
LEEP / LLETZ	loop electrosurgical excision procedure
LSIL	low-grade squamous intraepithelial lesion
NETZ	needle excision of the transformation zone / needle conization
OR	odds ratio
pPROM	premature prelabor rupture of the fetal membranes
PPV	positive predictive value
RCT	randomized controlled trial
SCJ	squamocolumnar junction
SIR	standardized incidence ratio
SMR	standardized mortality ratio
STAKES	national research and development centre for welfare and health (nowadays THL)
TBS	the Bethesda system
THL	National Institute for Health and Welfare
TZ	transformation zone
WHO	World Health Organization

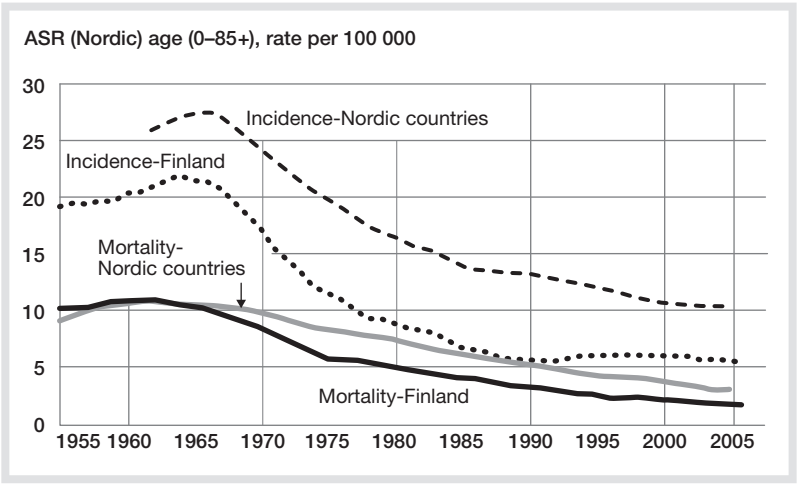
5 Introduction

Cancer of the uterine cervix (cervical cancer) is the third most common cancer among women worldwide. Over 85% of new cervical cancer cases occur in developing countries, where it is in many regions the most common cancer among women, constituting up to 13% of all new cancer cases. (Ferlay et al 2010). In Finland, an organized screening program for cervical cancer was launched regionally in 1963 and became nationwide in the early 1970's (Hakama and Räsänen-Virtanen 1976, Anttila et al 1999). Like other countries with a long history of organized screening programs for cervical precancerous lesions, age-adjusted cervical cancer incidence and mortality rates have been reduced by up to 80% in Finland since its introduction of the mass screening program (Hakama and Räsänen-Virtanen 1976, Hakama 1982, Hristova and Hakama 1997, Anttila et al 1999).

Even though nowadays it is very clear that for development of cervical cancer the Human Papilloma Virus, HPV, is essential, it is neither the sole nor a sufficient cause of the disease. Other factors playing their part in development of the disease include low socio-economic status, marital status, early age of sexual debut, use of oral contraceptives, alterations in the immune system (HIV infection), high number of sexual partners, multiparity, Chlamydia Trachomatis infections, and tobacco smoking (International Agency for Research on Cancer 2005). All these factors not only contribute to the development of cervical cancer: They are risk fac-

tors for other cancers (HPV, smoking) as well, they can be considered as determinants of health-care consumption and even life expectancy in general (socio-economic status, age), they can predispose to other morbidity (smoking for cardiovascular diseases) and to reproductive health complications and fertility (smoking, genital infections). Women treated for CIN are not only CIN patients; They possess a cluster of risk factors for other medical conditions, which may later cause other morbidity, and influence their overall survival.

Figure 1 Uterine Cervix. Time trends in cervical cancer incidence and mortality in Finland and in all Nordic countries.



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6 Review of the literature

6.1 Cervical intraepithelial neoplasia

6.1.1 Definition

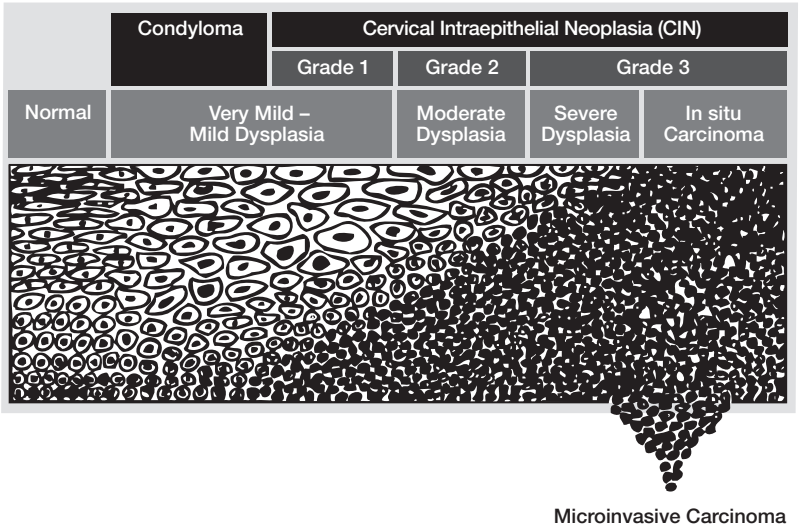
Most cervical cancers are derived from the epithelial tissue of the uterine cervix, meaning that they are carcinomas. The epithelium of the uterine cervix comprises both squamous and columnar epithelium, separated by a transformation zone, (TZ), where the columnar epithelium is slowly replaced through metaplasia during several years by stratified squamous epithelium. The border between these two epithelium types is called the squamocolumnar junction (SCJ). Most squamous-cell dysplasias develop within the transformation zone and most glandular carcinomas inside the cervical canal near the SJC. Squamous cell cervical cancer develops through premalignant intraepithelial lesions, dysplasias called cervical intraepithelial neoplasias (CIN). The only well-known precursor of cervical adenocarcinoma is adenocarcinoma in situ, (AIS), whose natural course is poorly understood (International Agency for Research on Cancer 2005). A histopathological diagnosis of cervical intraepithelial glandular neoplasia (CIGN) grades 2–3 also exists (Gloor and Hurliman 1986).

At the cellular level, a combination of disturbed cellular maturation, nuclear and cytoplasmic polymorphism, and increased cellularity is observable in CIN lesions. These premalignant cells already present with

several malignant features, such as cellular overcrowding, hyperchromatic nuclei, and nuclear polymorphism. These cells are intraepithelial, strictly restricted to the epithelium: The basement membrane is not breached, and no features characteristic for cancer such as infiltrative or metastatic growth exist (MacSween and Whaley 1992, Tavassoli and Devilee 1993).

Cervical intraepithelial neoplasias are graded in three categories: CIN 1, CIN 2, and CIN 3, depending on the thickness of the epithelium harbored by the dysplastic epithelial cells. The CIN 1 lesion is restricted to the lowest third of the epithelium, CIN 2 constitutes two-thirds of the epithelium, and CIN 3 affects the whole depth of the epithelium (MacSween and Whaley 1992).

Figure 2 Epithelial changes in different levels of dysplasia or CIN.



6.1.2 Etiology

Persistent or recurrent infection with high-risk human papilloma virus (hrHPV) types is a necessary, but not sufficient cause of CIN and cervical cancer (zur Hausen 1976, Munoz et al 1992, Bosch et al 1995, Zur Hausen 2000). HPV DNA can be detected practically always in cancer tissue (Clifford et al 2003, Munoz et al 2003). Human papillomaviruses are

double-stranded, non-enveloped DNA viruses that infect differentiating epithelial cells of the skin and mucosa (International Agency for Research on Cancer 2007). Over 130 different HP viruses have been identified (International Agency for Research on Cancer 2007), and approximately 40 types of them are capable of infecting the anogenital area (de Villiers et al 2004). These viruses are again classified into low- and high-risk types according to their association with various cervical lesions: The former are mainly involved in development of genital warts and mild dysplasia, and the latter in the development of malignant neoplasia. The most common hrHPV types, 16 and 18, are detected in approximately 70% of all cervical cancers worldwide (Munoz et al 2004). Other high-risk HPV types include 33, 45, 31, 58, 52, 35, 59, 56, 51, 39, 73, 68, and 82, in their order of decreasing worldwide prevalence (Clifford et al 2003, Munoz et al 2003). Their carcinogenic potential varies significantly, and a recent review concluded that HPV types 45, 31, 33, 35, 52 and 58, together with 16 and 18, are the most important hrHPV types globally in terms of carcinogenic potential. (Schiffman et al 2009)

In addition to HPV, smoking, early sexual debut, multiparity, high number of sexual partners, smoking, other genital infections — especially Chlamydia Trachomatis, human immunodeficiency virus (HIV), use of oral contraceptives, and low socio-economic status are known risk factors for CIN and squamous cell cervical cancer (Terris et al 1967, Koutsky et al 1992, Deacon et al 2000, Anttila et al 2001, Castellsague et al 2002, Smith et al 2004, International Agency for Research on Cancer 2005, Castellsague 2008). Apart from smoking, the risk factors for both squamous cell and columnar cell cervical carcinoma are identical (Berrington de Gonzales et al 2004).

The association between smoking and CIN is well documented: CIN patients are more likely to smoke more often than does the general population (Vaccarella et al 2008), and the current opinion is that smoking is an independent risk factor for cervical intraepithelial neoplasia (Burger et al 1993, Kjellberg et al 2000) and for squamous cell cervical cancer (Plummer et al 2003, Appleby et al 2006, Kapeu et al 2009).

Development of CIN has also been associated with the use of oral contraceptives. The relative risk for CIN among oral contraceptive users is increased and declines after cessation of usage (Moreno et al 2002, Smith

et al 2003, Appleby et al 2007). It is still difficult to differentiate whether oral contraceptive use is directly associated with CIN development, or whether oral contraceptive use elevates the rate of new or recurrent HPV infections and therefore of CIN incidence. Supporting this theory of indirect association, a study by Castle et al (2005) concluded that "Oral contraceptive use, Norplant® (implantable hormonal contraceptive) use, a history of pregnancy, age at first pregnancy, lifetime numbers of pregnancies and lifetime numbers of live births were not associated with CIN 3, when CIN 3 incidence was analyzed only from women with high grade HPV" (Castle et al 2005).

Because CIN is caused by infection, women with HIV or other immunosuppressive conditions are at increased risk for cervical cancer and its precursors (Birkeland et al 1995, Frisch et al 2000, International Agency for Research on Cancer 2005).

6.1.3 Natural history of HPV infection

Sexual transmission is the dominant route of acquiring anogenital HPV infection (Ley et al 1991, Bauer et al 1993, Rylander et al 1994, Franco 1995, Dillner et al 1999), and usually the infection is acquired at a young age, within a few years of the sexual debut (Koutsky et al 1992, Melkert et al 1993, Ho et al 1998, Rodriguez et al 2007). Condom use does not protect against HPV infection (Manhart and Koutsky 2002), but the incident HPV infections were 70% less common among regular condom users than among those who used condoms in less than 5% of the time, HR 0.5 (95% CI 0.1–0.6) (Winler et al 2006). In a Finnish study, genital HPV appeared in 15% of all infants at birth, declining to 10% at 24 months, with oral HPV in the mother as a risk factor for this (Rintala et al 2005).

Most HPV infections are transient, and they clear within months. Median duration of HPV infection among young women aged 13 to 23 was 8 months, and the cumulative 36-month HPV incidence of being HPV-negative has been 43%. Increasing age and infection with multiple HPV types has been associated with lower rate of HPV clearance from the cervix. (Ho et al 1998). Most infections are cleared by the immune

system within a few months, but hrHPV infections, especially HPV 16-infections, last a couple of months longer than do infections from low-risk HPV types (Richardson et al 2003). In one Finnish cohort, a third of a population of university students had a prevalent HPV infection, and 84% of these were hrHPV infections (Auvinen et al 2005). In Finland, the prevalence of high-risk HPV infection among women at screening age (30–65 years) is about 7% (Syrjänen et al 1992, Leinonen et al 2009). Over 50% of all non-specific HPV-related changes in cytological examination (ASC-US-LSIL) regress spontaneously without treatment (Melnikow et al 1998, Moscicki et al 2001, 2004). Infection with high-risk HPV persisting for over 6 months leads to an increased probability of developing dysplasia (Ho et al 1998, Moscicki et al 2001).

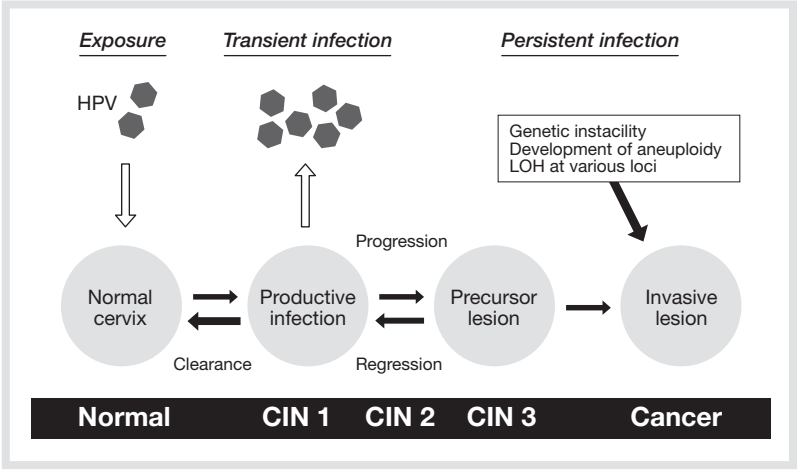
According to current knowledge, cervical cancer develops through precancerous lesions preceded by a persistent HPV infection (Koutsky et al 1992, Ho et al 1995, Remmink et al 1995, Ho et al 1998, Nobbenhuis et al 1999). Persistent HPV infections in the TZ of the cervix can result in either productive, self-limited HPV infections, or in infections with the potential to progress to invasive squamous cell carcinoma (Wright et al 2002). A typical cytological sign of productive HPV infections of the cervix is a low-grade squamous intraepithelial lesion (LSIL), and the corresponding histological lesions include condyloma and mild dysplasia (CIN 1). Histological lesions with the potential to progress to cervical cancer include moderate dysplasia (CIN 2), severe dysplasia, and carcinoma in situ (CIN 3). The corresponding cytological lesion for these is a high-grade squamous intraepithelial lesion (HSIL) (Wright et al 2002).

In transitional cervical infections, the HPV DNA remains episomal, but especially in CIN 3 and invasive cancer lesions the HPV DNA can become integrated into the host genome (Boshart et al 1984, Schwartz et al 1985), and this can result in genetic instability, secondary somatic mutations, and growth advantage in these cells with integrated HPV DNA (Jeon et al 1995, Zur Hausen 2000). Several studies have found only episomal HPV DNA in CIN 3 and cervical cancer cells (Fuchs et al 1989, Cullen et al 1991, Pirami et al 1997). The role of the HPV genome integration in cells therefore remains somewhat controversial.

6.1.4 Natural History of CIN

All CIN lesions are dynamic. They regress, persist, and progress at different rates over time, depending on the age of the woman, the grade of the lesion, persistence of the HPV infection, and on other risk factors such as smoking (International Agency for Research on Cancer 2005). Due to this and the variable endpoints used, CIN progression and regression rates vary significantly between studies.

Figure 3 Cervical cancer development.



Generally over 90% of all grades of CIN regress spontaneously among women aged under 22 (Moscicki et al 2004). This probability of spontaneous regression decreases with increasing age: Among women under 34, 84% (CI 76%–92%) of the new lesions (CIN 1–3) are estimated to regress spontaneously, but among women over the age of 34, only 40% of the new lesions seem to regress spontaneously (van Oortmarssen and Habbema 1991). Of cervical carcinoma in situ, 61% regressed spontaneously among women age 40 to 64, 70% among women 25 to 54, and 77% among women 15 to 39 (Boyes et al 1982). In an RCT performed in the Netherlands, women with an unspecified grade of CIN were assigned to regular condom use for at least 3 months or to a control group. The cumulative 2-year

regression rate of CIN was 53% among the condom users and 35% in the non-condom group (p= 0.03) (Hogewoning et al 2003).

Most low-grade lesions regress spontaneously, and progression rates into cancer are estimated to be very small. In a cohort of 528 women with LSIL or HSIL in a PAP smear, progression to CIN 2 or higher over the follow-up of 6 years in a Finnish cohort study was 14% among untreated women (Syrjänen et al 1992). The CIN 2 lesions progress more often, and in the same cohort the progression rates for CIN 2 and CIN 3 (with expansion of the detected CIN 3 lesion interpreted as progression of CIN 3) were 21% for CIN 2 and 69% for CIN 3 in the 6 years of follow-up (Syrjänen et al 1992). In this same cohort, 47% of women with initial LSIL and 38% of women with initial HSIL were under age 24 at the beginning of follow-up. The regression rate in this cohort for CIN 2 was 53% and for CIN 3, 14%.

In a Canadian historical cohort study of the PAP smear and biopsy history of women treated for CIN, the progression rates from CIN to cancer for CIN 1 was 0.4% (95% CI 0.3–0.5%), for CIN 2, 1.2% (CI 0.9–1.5%), and for CIN 3, 3.9% (CI 2.0–5.8%) (Holowaty et al 1999). The suggested progression rates of carcinoma in situ to cervical cancer range from 28 to 39% based on Finnish Cancer Registry data on cervical cancer incidence in a screened population, (Hakama and Räsänen-Virtanen 1976), to 15 to 23% according to a Swedish population-based study on invasive cancer and cervical carcinoma in situ incidence between 1958 and 1981 (Gustafsson and Adami 1989), and to 36% in a literature review (Mitchell et al 1996). A cohort study from New Zealand, a retrospective nationwide follow-up with 14% of women lost to follow-up, estimated that among women with untreated CIN 3, 20.0% (CI 13.7–28.7%) developed invasive cervical or vaginal cancer within 10 years and 31.3% (CI 22.7–42.3%) within 30 years (McCredie et al 2008).

Several studies have estimated the CIN grade-dependent progression and regression rates over the years. In one of the most prominent, a review by Östör (1993), the duration of follow-up and age at treatment were not considered in detail, and therefore the study is very likely to underestimate the real long-term progression rates from CIN into cancer (International Agency for Research on Cancer 2005, European Guidelines for Quality Assurance in Cervical Cancer Screening 2008) and this is not therefore further considered here.

The progression from hrHPV infection to cervical cancer takes decades. The time from HPV infection to carcinoma in situ is estimated to be at least 7 to 9 years (Ylitalo et al 2000b), the average duration of the dysplasia and carcinoma in situ stage has been estimated to be 11.8 years (van Oortmarssen and Habbema 1991); the duration of the carcinoma in situ stage alone has been suggested to be at least 5 to 10 years (Kasper et al 1970, Prorok 1986), however.

A model-based study using some of the progression and regression estimates referred to above estimated the lifetime incidence of cervical cancer in an unscreened population to be 3.67% with a lifetime cervical cancer mortality risk of 1.26%, They concluded that, based on the model, " the incidence of HPV infections, the proportion of rapidly progressive infections, and low-grade SIL progression rates appear to have the largest impact on cervical cancer risk," highlighting the possible effectiveness of primary prevention modalities against cervical cancer (Myers et al 2000).

6.2 Diagnosis and treatment of CIN

6.2.1 Diagnosis

In Finland about 850 women are annually diagnosed with CIN 3, and about 150 with cervical cancer (Finnish cancer registry 2010). No nationwide register of CIN 1 and 2 lesions exists. The incidence of CIN 3 lesions in the Finnish Cancer Registry might well be an underestimation due to poor compliance in notifying the detected CIN 3 lesions to the Finnish Cancer Registry. Early detection and treatment of HPV-related precancerous lesions, CINs, by nationwide screening has reduced the cervical cancer incidence and mortality in Finland by 80% since introduction of screening in the early 1960's (Läärä et al 1987, Anttila et al 1999, International Agency for Research on Cancer 2005). Since the 1990's, however, the incidence of cervical cancer has increased in Finland, especially among the youngest screening cohorts, women aged 30 to 39 years (Finnish Cancer Registry). The reason for this is both the low attendance rate at screening among these younger women as well as the simultaneous increase in HPV 16 incidence and seroprevalence in the same age cohort (Laukkanen et al 2003, Harper et al 2010)

The detection of cervical precancerous lesions has been based on exfoliative cytology, traditionally on the PAP smear, named after its inventor, Georgios Papanicolaou (Papanicolaou 1928, Papanicolaou and Traut 1941). It consists of three individually scraped samples of the vagina, cervix, and endocervix, all collected on the same microscope glass slide. The sample is then immediately fixed and stained with modified Papanicoloau staining. Within the nationwide screening-program, the sample is prescreened by a cytotechnician and after that examined by a cytopathologist. The current cytological terminology recommended for use worldwide to describe findings in the PAP smear is The Bethesda System, from the year 2001, (TBS 2001). It evaluates the adequacy of the specimen, gives a descriptive diagnosis, and distinguishes between intraepithelial atypia and infectious or reactive changes (Solomon et al 2001). All these features were missing from the original five-step Papanicolaou class report of the specimen (Papanicolaou 1954) (Table 1).

Table 1 TBS 2001 and CIN classifications of squamous cell lesions according to Papanicolaou class.

Papanicolaou	I	II	III	IV	V
TBS 2001	Negative for epithelial abnormality	ASC-US + LSIL	LSIL + HSIL	ASC-H & HSIL	Invasive SCC
CIN	Normal	Atypia	CIN 1,2 & 3	CIN 3	Invasive SCC

CIN = cervical intraepithelial neoplasia; SCC = cervical squamous cell cancer; TBS 2001 = the Bethesda System 2001; ASC-US = atypical squamous cells of unknown significance; LSIL = low-grade squamous intraepithelial lesion; HSIL = high-grade squamous intraepithelial lesion; ASC-H = atypical squamous cells, HSIL not excluded.

According to Finnish legislation, communities must organize mass screening for cervical cancer for women aged between 30 and 60 at 5-year intervals. Currently the screening is PAP smear-based, but recently HPV test-based screening has been suggested as the primary screening method due to its higher sensitivity in CIN detection (Leinonen et al 2009, Sankaranarayanan et al 2009, Anttila et al 2010, Ronco et al 2010).

The diagnostics of cervical epithelial cellular alterations is currently based mainly on cytology. Specific guidelines have been assessed, according to which women with different cytologically detected changes are

referred for resampling or colposcopy during which histological samples (biopsies) are taken (Finnish Current Care guidelines 2010). The terminology for describing the histological grade of the precancerous lesion is called the CIN terminology (Richart 1968, 1973).

6.2.2 Colposcopy

According to Current Care Guidelines in Finland, cytological indications for colposcopy are:

- Macroscopic suspicion of cancer and carcinoma in a cytological sample
- AIS, HSIL, ASC-H, AGC-FN, and atypical columnar endometrial cells (when diagnosis is not reached with an endometrial sample and ultrasound examination)
- LSIL: for women over age 30. Women under 30 according to recommendation of a cytopathologist or when ASC-US or a more severe lesion is detected in the follow-up PAP smear taken 6 to 12 months after the initial PAP smear.
- ASC-US when repetitive 2 to 3 times within 12 to 24 months, or ASC-US and hrHPV positive and aged over 35.
- AGC-NOS, when detected twice within 4 to 6 months
- Strong regenerative cytology or inflammation when repeatedly discovered according to a cytopathologist's recommendation.

The colposcope was first described by Hinselmann (1925). The basic principle has remained the same to date; the colposcope is a binocular light microscope allowing the cervix to be magnified up to 40 times its normal size (Anderson et al 1996). The cervix is visualized with a speculum, and the colposcopic examination for abnormalities includes examination of the squamous epithelium, the TZ, the squamocolumnar junction, and the visible part of the columnar epithelium (Coppleson et al 1978). During the examination, a 3 to 5% acetic acid solution is applied to the cervix. This causes tissue swelling and coagulation of the superficial intracellular proteins that can be observed as reduced transparency and whitening of the epithelium (acetowhitening) (Anderson et al 1996).

The colposcopical examination and interpretation is based on visual patterns of acetowhitening and on vascular patterns. Changes in subepithelial angioarchitecture suggestive for CIN are punctuation, mosaic, and atypical vessels. High-grade lesions are more densely aceto-white than low-grade lesions, and the borders of high-grade lesions have sharp edges, whereas the edges in low-grade lesions can be indistinct. (European Guidelines for Quality Assurance in Cervical Cancer Screening 2008).

From the areas where the acetowhitening is most prominent — the most suspicious areas in terms of possible CIN — punch biopsies, more than one if necessary, are taken under colposcopical control. The biopsy must include both the surface epithelium and the stroma to indicate whether the lesion is intraepithelial. Usually no local anesthesia is required (Finnish Current Care guidelines 2010).

All acetowhite areas are not premalignant, and this phenomenon can be observed in immature squamous metaplasia, in healing or regenerating epithelium, in HPV infection, and in invasive carcinoma as well (European Guidelines for Quality Assurance in Cervical Cancer Screening 2008). This is the reason for generally low specificity of colposcopy, 48% on average for diagnosing any abnormality and 69% for diagnosis of high-grade (CIN 2 & CIN 3) or invasive lesions, compared to sensitivity of 96% for any abnormality and 85% for high-grade and invasive lesions (Mitchell et al 1998). In another study where biopsy specimens were taken not only from colposcopically suspect areas but also routinely from all quadrants of TZ with endocervical curettage, the sensitivity of CIN 2+ detection for colposcopy was 57% (95% CI 52–62%) (Pretorius et al 2004). A review of the literature estimated that the positive predictive value (PPV) for colposcopy is 78% in detecting CIN 3 lesions, and smaller in detecting CIN 1 and 2 lesions (Hopman et al 1998).

6.2.3. Management of CIN

Diagnosis of CIN 1 is not always reliable, and its histological reproducibility is poor (Stoler and Schiffman 2001). Moreover, CIN 1 lesions have a high probability of regression, especially among women under 30 (Moscicki et al 2004). Because of this, CIN 1 lesions can be followed for spontaneous regression for up to 12 to 24 months (European Guidelines for Quality Assurance

in Cervical Cancer Screening 2008, Finnish Current Care guidelines 2010). Finnish current care guidelines recommend that women be treated for CIN 1 after 24 months of persistence, regardless of their age (Finnish Current Care guidelines 2010). Historically, all CIN lesions have been treated in Finland.

CIN 2 and CIN 3 lesions are virtually always treated because of their higher probability of progression (European Guidelines for Quality Assurance in Cervical Cancer Screening 2008, Finnish Current Care guidelines 2010). Histologically verified precursors of cervical adenocarcinoma AIS and cervical intraepithelial glandular neoplasias, grades 2–3 (CIGN 2–3) are always treated. Moreover, diagnostic LLETZ is nearly always performed for cytologically detected high-grade glandular changes: for atypical glandular cells, favor neoplasia (AGC-FN) and for 24 months persistent low-grade glandular changes, i.e. atypical glandular cells not otherwise specified (AGC-NOS) (Finnish Current Care guidelines 2010). During pregnancy their treatment is recommended only in case of suspicion of invasion (Finnish Current Care guidelines 2010)

The treatment procedure is performed under colposcopical control, under local anesthesia, on an outpatient basis. The cervical epithelium is stained with acetic acid, and the whole treatment procedure is performed through a colposcope. The aim of the treatment procedure is to remove or destroy the whole TZ and the lesion within.

Excisional procedures are nowadays preferred in most circumstances because of the possibility to examine the removed tissue as a histological specimen, providing information about the success of the procedure and confirming the CIN grade. Guidelines recommend that excision be mandatory when the lesion extends into the endocervical canal, i.e. when the lesion is not fully visible or when a persistent or recurrent lesion is treated (Finnish Current Care guidelines 2010).

The complete TZ including the preinvasive lesion is excised. Excisional techniques have been:

- LLETZ, excision of the TZ using a diathermy loop; used in Finland nowadays nearly always
- CKC, removal of cervical tissue by means of a knife
- Laser excision, removal of cervical tissue with a CO2 laser in the cutting mode

- Needle excision, NETZ, excising the TZ with a straight diathermy wire

Current guidelines in the EU and Finland reserve the use of ablative techniques for lesions in which the entire TZ must be visible (European Guidelines for Quality Assurance in Cervical Cancer Screening 2008, Finnish Current Care guidelines 2010). The most common destructive method in Finland, cryotherapy, requires a double freeze-technique (Schantz and Thormann 1984). In addition, the lesion must occupy no more than 75% of the ectocervix, the lesion must not extend into the vaginal wall, to the endocervix, or more than 2 mm beyond the cryoprobe. There should be no evidence of invasive or glandular disease prior to the treatment, and prior to the ablative treatment, a biopsy is taken from most suspicious part of the lesion, and histological grade must correspond to the cytological grade of the lesion (European Guidelines for Quality Assurance in Cervical Cancer Screening 2008, Finnish Current Care guidelines 2010). When an ablative therapy is used, the TZ destruction should extend beyond the margins of the lesion and should be, at minimum, of a depth of 4 to 7mm.

The aim of local destructive / ablative therapy is to destroy the CIN. The techniques used are:

- Cryotherapy, in which a probe is applied to the tissue that is destroyed by freezing
- Laser vaporization, in which CO2 laser at high power vaporizes the water in the cell and destroys the tissue
- Radical diathermy, in which a straight electrodiathermy needle is applied and destroys the tissue to an approximately depth of 1cm

6.3 Short-term outcomes after CIN treatment

6.3.1 Treatment complications

After local treatment of CIN, immediate complications include perioperative pain and bleeding, secondary bleeding after the procedure, leucorrhea, infections, and cervical stenosis (Finnish Current Care guidelines 2010). These are generally uncommon, and no clear differences exist in the incidence of these complications between treatment methods (Larsson et al 1982, Berget et al 1987, Oyesanya et al 1993, Martin-Hirsch et al 2010). Pain is experienced by 2 to 18% of women, and disturbing bleeding occurs among 2 to 12% (Martin-Hirsch et al 2010).

6.3.2 CIN persistence and recurrence

Success rates, i.e. absence of recurrent or residual disease within a few years after LLETZ, CKC, laser conization, or laser ablation, range from 90 to 98% (Larsson 1983, Jordan et al 1985, Bostofte et al 1986, Tabor and Berget 1990, Luesley et al 1990, Bigrigg et al 1990, Martin-Hirsch et al 2010). For cryotherapy, success rates for treatment of CIN 3 range from 77% to 93% (Popkin et al 1978, Hatch et al 1981). Still, including all randomized controlled trials comparing all these techniques, no technique was superior to another in terms of success or failure rates, i.e. in terms of incidence of residual disease (Martin-Hirsch et al 2010).

Most persistent or recurrent CIN cases occur within 24 months of treatment (Flannelly et al 2001, Chew et al 1999). Recurrence rate of any CIN is elevated for 6 years after treatment of CIN; after that, no difference in CIN incidence appears between the treated and healthy women (Melnikow et al 2009). Age at treatment (over age 40) (European Guidelines for Quality Assurance in Cervical Cancer Screening 2008, Melnikow et al 2009), involvement of margins (incomplete excision) (Andersen et al 1990, Dobbs et al 2000, Flannelly et al 2001, Ghaem-Maghami et al 2007), high pre-conization hrHPV load (Park et al 2007), and presence of glandular disease (Soutter et al 2001) predict higher rate of CIN recurrence. Also high grade CIN and treatment with cryotherapy are clear risk factors for CIN recurrence (Melnikow et al 2009).

6.3.3 Follow-up

According to current care guidelines, all women treated for CIN must be followed up (Finnish Current Care guidelines 2010). After treatment of a CIN 1 lesion, a PAP smear is taken after 6 months, along with an hrHPV test and colposcopy when needed. If the PAP smear appears normal and the hrHPV test is negative, a new PAP smear is taken after 24 months post-treatment. If this sample again appears normal, the woman may return to normal screening intervals. If the first post-treatment PAP smear is abnormal, a new smear is required after an additional 6 months and then at 24 months post-treatment.

For women treated for CIN 2, CIN 3, or AIS, a colposcopy along with a PAP smear and an hrHPV test is performed 6 months after the treatment. If all three appear normal, the next PAP smear is taken 24 months after the treatment and then annually until 5 years after treatment. After this, the woman returns to 5-year interval screening. If any appears abnormal, a new colposcopy and PAP smear are performed after an additional 6 months. If only a PAP smear and colposcopy are performed at 6 months after treatment, a new PAP smear, and colposcopy when needed, is performed after 12 months. If they again appear normal, annual PAP smears are taken until 5 years after the treatment. After this the woman may return to normal screening intervals (Finnish Current Care guidelines 2010). These current care guidelines in Finland were first introduced in 2006 and updated in 2010. At the time of treatment for the women in our data, some variation may have existed in the follow-up after the CIN treatment.

6.4 Long-term outcomes after CIN treatment

From among studies concerning endpoints similar to ours, only those published before ours are considered here.

6.4.1 Cervical cancer incidence

Among 795 women treated for cervical carcinoma in situ with CKC, 7 women (0.9%) were diagnosed with invasive cervical cancer within 5 to 20 years after treatment (Kolstad and Klem 1976). A cancer registry-based study from Sweden estimated the cervical cancer incidence after CKC of cervical carcinoma in situ to be roughly 2.5 times as high for 20 years among the treated compared to the general population (Pettersson and Malker 1989). In a Swiss study in which women treated for cervical carcinoma in situ were compared to a general population, the standardized incidence ratio (SIR) for cervical cancer was 3.4 ($p<0.01$) (Levi et al 1996). Another study with cytological and hospital-based follow-up found the incidence of cervical cancer to be elevated regardless of treatment method and after treatment of CIN 1 or 2 as well, and concluded that the incidence of cervical cancer after treatment of CIN was 5.8/1 000 women or 85/100 000 woman years (Soutter et al 1997).

A study of 843 women treated with cryotherapy with follow-up of at least 5 years reported one cervical adenocarcinoma at 6 years, suggesting a cumulative rate of invasive cancer of approximately 1.2 per 1 000 women (Benedet et al 1987). A study of 1 053 women treated mainly for CIN 3 with laser conization showed a cumulative rate of invasive disease of 4 per 1 000 women by 6 years with 6 540 woman-years of follow-up time and an overall rate of 61 per 100 000 woman years (Skjeldestad et al 1997).

Soutter et al (1997) estimated the overall cervical cancer-preventive effect of CIN treatments to be 95%, based on progress rates of different grades of CIN from one unethical study, in which some women with CIN were left untreated and followed up for cancer incidence (McIndoe et al 1984).

6.4.2 Other than cervical cancer incidence

Some evidence of increased other-cancer incidence after treatment of CIN already existed before the beginning of our study. After treatment of CIN 3, especially the anogenital cancers, cancers of the vagina, vulva, and anus, which share the same risk factor HPV, were more common than in the general population (Hemminki et al 2000a). Increased risk for cancers of major tobacco-related sites (lung, mouth or pharynx, esophagus, and urinary bladder) were also increased after CIN 3 diagnosis (Levi et al 1996, Hemminki et al 2000a, Evans et al 2003). Cancers of the upper aerodigestive tract and pancreas (Hemminki et al 2000a), as well as non-melanoma skin cancers (Levi et al 1996) were increased after treated CIN 3 as well. Tonsillar cancer incidence was 2.4-fold among women over 50 at the time of CIN 3 diagnosis (Hemminki et al 2000b). No studies were found of other than cervical cancer incidence according to grade of CIN or method of treatment.

6.4.3 Mortality

A Finnish study by Hakama et al (2004) reported overall mortality to be higher among women treated for cervical carcinoma in situ than for the general population. The risk of death was increased only at advanced ages and was independent of age at diagnosis of carcinoma in situ. To our knowledge, no other studies on mortality after treatment of any grade of CIN exist.

6.4.4 Fertility and pregnancy outcome

Treatment of CIN can cause scarring of the cervix and removal of cervical mucus-secreting cells (Hammond et al 1990, Kennedy et al 1993). The scarring may result in cervical stenosis (Baldauf et al 1996) and prevent sperm from entering the uterus or cause alterations in the cervical mucus and therefore infertility. The loss of cervical mucus-secreting glands may compromise the cervical immune defense and thus predispose to

ascending infection and to premature prelabor rupture of the fetal membranes (pPROM) and even to tubal infertility (Hammond et al 1990, Fox and Cahill 1991). Cervical stenosis occurs in 2 to 37% of women treated for CIN, depending on the treatment modality (Martin-Hirsch et al 2010).

Studies of adverse pregnancy outcomes after treatment of CIN are vast. The two largest meta-analyses so far, both comparing incidence of adverse pregnancy outcomes between the treated women and the general population, concluded that excisional treatment methods (CKC, LLETZ, and laser conization) are associated with preterm delivery (<37 gestational weeks) and low birth-weight (Kyrgiou et al 2006, Arbyn et al 2008). LLETZ was also associated with premature prelabor rupture of the fetal membranes (pPROM), and CKC with increased incidence of caesarean section in successive pregnancies. No such associations were observed after the ablative treatment methods (laser vaporization and cryotherapy). CKC was also associated with increased perinatal mortality, and severe or extreme pre-term birth (Arbyn et al 2008). In that meta-analysis, LLETZ and ablative treatment methods were not associated with these serious adverse pregnancy outcomes (perinatal mortality, severe or extreme preterm birth, and birth-weight <2 000g) (Arbyn et al 2008). A more recent study, comparing incidence of pPROM and spontaneous preterm delivery after different treatment methods, found no differences between the methods used, however (Shanbagh et al 2009). The depth of cone removed has been associated with increased risk for spontaneous preterm delivery in recent studies (Noehr et al 2009, Jakobsson et al 2009B). The proportion of the volume of cervix excised varies significantly, and is directly associated with the proportional deficit cervix volume at 6 months (Founta et al 2010).

A retrospective cohort study from New Zealand compared deliveries of women treated for CIN to those of women referred to a colposcopy clinic but not treated for CIN. This study collected information about the smoking status, their socioeconomic status, number of pregnancies, and order of the current pregnancy; and differentiated between types of preterm delivery (spontaneous preterm delivery, pPROM, medical induction). They observed CIN treatment (LEEP or laser conization) not to increase the risk for total or spontaneous preterm delivery. After LEEP and laser conization, however, risk for pPROM was increased, especially with increasing

size of the cone (Sadler et al 2004). When women treated for CIN were compared to themselves before the treatment in terms of pregnancy complications, the treated women were at only slightly higher risk for pre-term birth (Bruinsma et al 2007). Furthermore, when all possible relevant confounding factors (history of one or more induced abortions, two or more miscarriages, illicit drug use during pregnancy, having a major maternal medical condition, being single, and being of older maternal age) were adjusted for, only needle diathermy, NETZ, was associated with increased incidence of pre-term birth (Bruinsma et al 2007).

A study using the Medical Birth Registry of Norway (all births between 1967 and 2003) concluded that women with CIN are at significantly increased risk for any pre-term delivery after the conization compared to the period before the conization (Albrechtsen et al 2008). No stratification between different methods of treatment or time-period was performed. A Finnish cohort of women treated with LLETZ identified similar findings of increased post-treatment risk for pre-term delivery (Jakobsson et al 2009B).

Because numerous studies, also using Finnish data, exist about the association between CIN treatments and pre-term delivery, we decided not to study that association. Instead, studies about possible infertility after CIN treatment are scarce and based on small samples. One older review reported no impairment in fertility after CKC (Weber and Obel 1979). A review concerning fertility after CIN treatment found no effect on future fertility (Hammond and Edmonds 1990). One study documented pregnancy incidence to be the same after LLETZ and in the general population (Ferenczy et al 1995). Conversely, women treated with laser vaporization or excision had more pregnancies and deliveries after than before that treatment (Spitzer et al 1995). Two studies about future fertility after LLETZ using a postal questionnaire concluded that the treatment had no effect on future fertility (Bigrigg et al 1994, Cruickshank 95). No increase was observed in IVF pregnancies after the treatment of CIN, also indicating the treatment did not compromise future fertility (Jakobsson et al 2008).

Nor has the miscarriage rate has increased among treated women (Weber and Obel 1979, Spitzer et al 1995, Tan et al 2004), but older studies of post-CKC pregnancies saw some increase in spontaneous first and second trimester abortions (Lee 1978, Jones et al 1979). Some evidence

about the association exists between increased rate of induced abortions and cervical cancer or dysplasia (Parazzini et al 1989, Wang and Lin 1995, Spitzer et al 1995). Spitzer et al (1995) concluded that women might have been worried about progression of their disease and therefore requested more induced abortions. Ectopic pregnancies were in one study not more common after than before laser ablation or excision of CIN (Spitzer et al 1995). The risk of late abortion was, however, increased threefold compared to that of a healthy population among women treated for CIN (Albrechtsen et al 2008).

Possible factors associated with fertility and parturition after a CIN diagnosis are the anxiety and distress associated with discovery and treatment of CIN (Marteau et al 1990, Le et al 2006). Concern about cancer, loss of attractiveness, loss of sexual functioning, anxiety, and low self-esteem are reported to exist both before and after colposcopy and punch-biopsy (McDonald et al 1989). One study of women referred to colposcopy and biopsy reported their "spontaneous interest in sex, frequency of intercourse, and sexual arousal to be statistically significantly lower at 6 months compared with the first visit, and at 2 years, spontaneous interest in sex and frequency of intercourse still remained low." (Hellsten et al 2008).

7. Aims of the study

The aim of this study was to evaluate long-term cancer incidence, mortality, and reproductive health among women treated for cervical intraepithelial neoplasia. Based on the results, we evaluated the efficacy and safety of CIN treatment practices, as well as the role of risk factors for other medical conditions of these women.

1. To determine the effectiveness of CIN treatments in terms of differences in cervical and other cancer incidence among women treated for CIN, with particular emphasis on HPV- and smoking-related primary sites.
2. To determine whether histopathological grade, age at treatment, and method of treatment affect the success of the treatment in terms of disease-free survival after subsequent CIN 3, cervical cancer, or other cancers.
3. To determine the efficacy of the CIN treatments by studying possible differences in cervical cancer mortality between the treated and the reference cohort, and to determine the influence of the other risk factors of women with CIN in terms of HPV- associated cancer, other cancers, and overall mortality.
4. To evaluate whether treatment of CIN has any effect on future fertility and pregnancy outcomes, i.e. whether there exist differences

in becoming pregnant and in giving birth, and in the incidence of induced abortions, or of abnormal pregnancy (spontaneous abortion, extra-uterine pregnancy, or molar pregnancy) between women treated for CIN and their reference population.

Studying all these long-term outcomes among women treated for CIN would help with decisions as to whom to treat and when, for how long, and how to follow up after treatment. Most of all, do these treatments prevent cancer and reduce mortality, and are they safe for the women in terms of future fertility?

8 Materials and Methods

8.1 Data sources, data work and study populations

All four studies are based on data concerning women treated for CIN at Helsinki University Central Hospital, Finland. This hospital was a major reference center in the Helsinki-Uusimaa region for women referred to colposcopy between 1974 and 2001. For each patient name, a unique personal identifier (PID), date and method of treatment (cold knife conization, cryotherapy, laser conization or vaporization or LEEP), and diagnosis on the basis of histopathology (CIN 1–3 or CIN NOS, CIN NOS for dysplasias diagnosed before the grading to CIN 1–3) came from hospital records. The women included had squamous cell cervical lesions treated with these conservative methods.

This primary data included a total of 22 985 visits of 7 600 patients and was further screened for any possible double visits (same visit recorded twice) or inadequate social security numbers. After this, the data comprised 22 939 visits or treatments of 7 599 women. These data were linked with the Finnish population registry to assure the correctness of social security numbers, to determine the possible time of death, and the possible date of emigration. Further excluded were 19 (0.25%) patients and 42 visits (0.18%), leaving 7 580 women for the final study population. We chose to use date, diagnosis, and method of treatment at the first visit for each

woman in all further analyses due to insufficient hospital data regarding possible follow-up visits. The research protocol of this study has been approved by the Ethics Committee, Section for Obstetrics and Gynaecology in the Helsinki-Uusimaa Hospital District.

8.1.1 Study I

The final study population, including 7 580 women and 22 898 visits, was linked to the Finnish Cancer Registry to identify cases of cervical cancer, other gynecological cancers, and any other cancers. In this procedure a further 16 patients were excluded due to a follow-up time of less than 6 months, leaving a total of 7 564 patients for the cancer analysis (Table 2).

The treatment for CIN was cold knife conization (CKC), laser vaporization or conization, cryotherapy or LEEP, depending on year of treatment. The histopathological diagnosis at the treatment was CIN 1 (n=2 446), CIN 2 (n= 1 543), CIN 3 (n=1 334), or CIN not otherwise specified (CIN NOS) (n=2 241).

The follow-up of cancer incidence began 6 months after the first visit, and lasted until death, emigration, or 31 December 2003. We set the lag period of 6 months before possible diagnosis of invasive cervical cancer to exclude cancers already present at the initial visit.

Table 2 Number of women by age group according to duration of follow-up time in woman-years.

Age group	Number of patients	Time of follow-up (woman-years)			
		0.5–9	10–19	20+	Overall
< 29	3 114	14 018	424	–	**14 441**
30–44	2 958	26 829	15 600	795	**43 225**
45–59	1 074	10 611	12 554	4 303	**27 468**
60–74	347	3 704	4 572	1 801	**10 077**
75+	71	773	1 142	430	**2 345**
Total	**7 564**	**55 934**	**34 291**	**7 330**	**97 556**

8.1.2 Study II

The primary data from Study 1, the 7 564 women treated for CIN, were linked with the updated Finnish Cancer Registry. Women diagnosed with invasive cervical, vaginal, vulvar or lung cancer before, or within 180 days of initial treatment, were excluded from the final analysis. Women who died or emigrated before or within 6 months after diagnosis were also excluded, comprising a further 98 women. Thus 7466 patients entered the survival analysis (Table 3).

The follow-up of cancer incidence and overall survival started 6 months after CIN treatment and lasted until the date of cancer diagnosis, or the date of death, the 31st December 2003, or at the date of emigration. The proportional hazard assumption was tested (Schonfeld 1982) for both specific variables and globally.

Table 3 Number of women according to age at diagnosis, initial histopathological grade of CIN, and method of treatment.

Age at diagnosis	Number of women	Follow-up years
0–15	7	89
16–30	3 411	47 784
31–45	2 744	36 159
46–60	959	12 434
61–75	294	3 442
76+	51	377
Grade of CIN	**Number of patients**	**Follow-up years**
CIN 1	2 440	34 383
CIN 2	1 541	11 199
CIN 3	1 258	13 971
CIN NOS	2 227	40 731
Method of treatment	**Number of patients**	**Follow-up years**
CKC	724	18 566
Cryo	488	11 181
Laser	3 104	53 464
LEEP	3 150	17 072
Total	7 466	100 284

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8.1.3 Study III

The primary data, including dates of death or emigration from the woman's first linkage to the Finnish population registry, was again linked to the Finnish population registry to retrieve a reference population of five control women for each woman treated, individually matched by

age and municipality at the time of CIN treatment. Birth dates of children were also gathered simultaneously when available. To identify cases of cancer, the whole data set, including the reference population, was further linked to the Finnish Cancer Registry.

Socioeconomic status came from Statistics Finland's census records between 1970 and 2004 for all women. During that period the census was performed every 5 years, and the social class in the census performed nearest to the time of treatment was set as the social class for the final analysis. Classification of socio-economic status varied slightly over time, so occupational social classes were divided into six common non-hierarchical classes used at all points of measurement: (1) upper white collar, including entrepreneurs, employers and agricultural entrepreneurs, and employers (2) lower white collar, (3) blue collar workers including agricultural workers (4) pensioners, (5) students over age 16, (6) housewives, the unemployed, and social class unknown. Children under were classed according to their parents.

Causes of deaths in the data were gathered from Statistics Finland's records from 1974 to 2005. The same ICD8-10 based longitudinal classification into 53 different possible causes of death was used throughout the study period. Deaths from cancers of the vagina, vulva, and anus were studied through Finnish Cancer Registry records because the classification retrieved from Statistics Finland did not include these as separate causes of death. Deaths from cervical and uterine cancer were also further verified by use of linkage with the Finnish Cancer Registry.

Women with cervical cancer diagnosed before or within 6 months after the treatment of CIN, women whose socioeconomic status could not be retrieved, and women for whom no controls were available were excluded from the final data set. When a treated woman was excluded, her controls were excluded as well. After all exclusions, 7 104 women treated for CIN and 35 437 reference women were included in the final data set (Table 4).

The follow-up of mortality started for both the treated and reference population on the day the CIN was treated and ended at death or emigration, or on December 31, 2005. Based on a potential association with CIN, we grouped all 56 possible causes of death into 23 categories. We put extra emphasis particularly on the HPV- and smoking-related causes of death, and estimated the most common causes of death as well.

Table 4 Number of women in the treated and the reference cohort.

	Number of CIN patients	Number of reference population	Median age at the start of follow-up	Time of follow-up	Average time of follow-up
Total	**7 104**	**35 437**	**32**	**628 071**	**14.8**
Grade of CIN					
CIN 1	2 229	11 111	30	199 134	14.9
CIN 2*	3 700	18 478	32	343 946	15.5
CIN 3	1 175	5 848	35	84 990	12.1
Age at treatment					
14–39	5 029	25 096	29	446 834	14.8
40–59	1 689	8 431	46	153 409	15.2
60–89	386	1 910	66	27 828	12.1
Socioeconomic status					
1	1 103	6 438	35	104 815	13.9
2	3 077	14 557	32	273 984	13.5
3	1 301	5 781	32	115 355	16.3
4	470	2 415	63	35 545	12.3
5	623	3 612	24	60 555	14.3
6	530	2 634	31	37 817	12

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*CIN NOS is included in CIN 2.

8.1.4. Study IV

For the fertility study, the primary data consisting both of women treated for CIN and the reference population were linked with THL (National Institute for Health and Welfare) Care Registers for Social Welfare and Health (previously called STAKES Hospital Discharge Register) to retrieve the precise times of other pregnancy outcomes (spontaneous abortions, extra-uterine pregnancies, molar pregnancies, and induced abortions). Live births were already retrieved from the Finnish population registry. Information on possible sterilization came from a separate THL Steriliza-

Table 5 Number of women overall and according to pregnancy outcomes after and before the treatment of CIN.

	N	pregnancies after treatment	pregnancies before treatment	deliveries after treatment	deliveries before treatment	spontaneus abortions after treatment	spontaneus abortions before treatment
Total*	36 615	26 335	38 963	21 174	30 900	941	1 009
Reference population	30 436	21 363	32 021	17 414	25 700	770	816
Treated women	6 179	4 972	6 942	3 760	5 200	171	193
CIN 1	1 983	1 687	2 006	1 284	1 493	66	64
CIN 2	3 183	2 760	3 941	2 073	2 587	94	94
CIN 3	1 013	525	1 445	403	1 120	11	35
CKC	414	198	657	121	618	13	3
Cryo	399	451	444	313	351	27	7
Laser	2 654	2 834	2 894	2 121	2 172	110	79
LEEP	2 712	1 968	2 947	1 205	2 059	21	104

	extra uterine pregnancies after treatment	extra uterine pregnancies before treatment	molar pregnancies after treatment	molar pregnancies before treatment	induced abortions after treatment	induced abortions before treatment
Total*	374	474	295	196	3 551	6 384
Reference population	267	369	242	158	2 670	4 978
Treated women	107	105	53	38	881	1 406
CIN 1	27	26	12	10	298	413
CIN 2	75	59	35	18	483	733
CIN 3	5	20	6	10	100	260
CKC	7	2	0	0	57	34
Cryo	18	8	2	1	91	77
Laser	79	51	38	5	486	587
LEEP	3	44	13	32	247	708

* Total means overall number of women both in treated and the reference populations

tion Registry, and induced abortions furthermore from the THL Abortion Registry. Data on all these pregnancy outcomes was reliably available only from January 1, 1974 forwards. Only singleton pregnancies were included in this study due to the differing nature of multiple fetuses.

We estimated the onset of pregnancy by median estimates of the duration of pregnancy for the outcome in question: 1) delivery; 280 days before the actual birth date, 2) spontaneous abortion and induced abortion; 63 days before, 3) extra-uterine pregnancy; 56 days before, 4) molar pregnancy; 77 days before.

Women over age 50 at the time or sterilized before the CIN treatment were excluded from all analyses. The data were again linked to the Finnish Cancer Registry to exclude all women diagnosed with invasive cervical cancer before or within 6 months after the initial treatment of CIN. Altogether 233 women with cervical cancer meeting these conditions were excluded during this process. Women under 15 at the time of treatment entered the follow-up when they turned 15. After all exclusions, 36 615 women (6 179 women treated and 30 436 control women) entered the statistical analysis (Table 5).

8.2 Statistical analysis

8.2.1 Study I

The possible differences in cervical and other cancer incidence between the treated women and the general population were calculated by comparing ratios of observed numbers to expected numbers of cancer cases (standardized incidence ratio, SIR). The 95% confidence intervals were calculated presuming that the number of cases observed followed a Poisson distribution. The expected numbers of cancer cases were stratified by sex, 5-year age groups, and 5-year calendar periods, based on the cancer incidence rates of southern Finland.

Cancer endpoints for the analysis were selected before the start of analysis, based on current knowledge of the etiology of cervical lesions. The risk for overall cancer, breast cancer, gynecological cancers, and of major smoking-related cancers, lung cancer, cancers which strongly correlate

with smoking (cancers of the larynx, tongue, mouth, other larynx besides naso- or hypopharynx, pancreas, bladder, and kidney) were calculated.

8.2.2. Study II

Differences in disease-free survival or overall survival between the treatment methods or CIN grades were assessed using the Cox proportional hazards regression model (Cox 1972).

The main outcomes studied were cervical cancer and the two other gynecological cancers (vulva, vagina) with a similar etiologic risk factor (HPV). We also estimated CIN 3, CIN 3+, overall cancer, and lung cancer-free survival, as well as overall survival. To control for confounding factors, all models were adjusted for age of woman at treatment and further stratified for the method of treatment and the grade of CIN.

8.2.3. Study III

We used the Cox proportional hazards model (Cox 1972) to assess the differences in survival between women treated for CIN and the reference population. Each matched set was defined by one woman treated for CIN and her five controls, matched with respect to age and place of residence at the beginning of follow-up. We compared hierarchical models using log-likelihood ratio statistics and tested the proportional hazard assumption (Schonfeld 1982), both for specific variables and globally for the final models. If the proportionality assumption was not fulfilled, a stratified Cox analysis with respect to socioeconomic status was performed, which always led the model fulfilling the assumption.

Final models were adjusted for socio-economic status, method of treatment, and grade of CIN.

8.2.4. Study IV

In all analyses, the incidence of deliveries, spontaneous abortions, extrauterine pregnancies, molar pregnancies, induced abortions or any type of

pregnancy (fertility) were calculated over the given follow-up time for the treated women and their reference population. The differences between risks were reported as hazard ratios (HR), and p-values less than 0.05 were considered significant.

The endpoints in the analysis were defined as follows: delivery means delivery over 24 weeks of gestation of a live-born baby; spontaneous abortion means spontaneous abortion before 23 gestational weeks; extra-uterine pregnancy means pregnancy outside the uterus, e.g. in fallopian tube, ovary, or abdominal cavity; molar pregnancy means benign gestational trophoblastic neoplasia; and induced abortion means medically performed abortion, either with medication or by dilatation and curettage. Fertility denotes incidence of all endpoints: deliveries, induced abortions, extra-uterine pregnancies, spontaneous abortions, and molar pregnancies.

We performed the main analysis of the data using a stratified Cox-regression model (Cox 1972). Follow-up began on the day of CIN treatment and ended 1) at the start of the first-ever post-treatment pregnancy (referred to later as COX1 analysis), 2) at the onset of first pregnancy of a particular type (e.g. the first extra-uterine pregnancy or the first delivery), regardless of how many post-treatment pregnancies preceded the first particular type of pregnancy (referred to later as COX2 analysis), or at sterilization, turning 50, emigration, death, or 31 December 2004. The analysis of all post-treatment pregnancies was performed with Poisson regression (Breslow and Day 1987) with the same conditions for follow-up.

In order to control for any possible confounding effect on their post-treatment fertility, number of any pregnancies (0, 1, 2, 3+) and children (0, 1, 2, 3+) before treatment for CIN, and whether the type of pregnancy in question had already occurred before the treatment were included in the models. Separate models specific to age at treatment (15–29 and 30–50 years), number of children before treatment (0 and 1+), grade of CIN (CIN 1–3), and method of treatment (CKC, cryotherapy, laser, LEEP were also performed). For Poisson models, extra adjustment was made for municipality and age at time of CIN treatment, and by year of treatment. Separate models were also fitted specifically to the number of children (0/1+) before CIN treatment. To monitor for any possible change in the hazard ratios over time, Cox models with different lengths of follow-up

were fitted as well.

We fitted a Cox regression model also for a subset of data, women born 1958 or later. In this subset, all pregnancies before the treatment possibly influencing their future fertility were included, because data on reproductive endpoints in the registers used were available from January 1, 1974. Because these results were very similar to the results with the whole data set, the whole data set was used for all analyses.

To monitor whether fertility rates between treated women and their reference population differed before treatment of CIN, a Poisson regression model of the number of different sites of pregnancy before the treatment was performed. The follow-up began when the woman turned 15 or on January 1, 1974 (no information on pregnancies prior to this date were available) and ended at the date of CIN diagnosis. All models were adjusted for the woman's age, municipality, and the year of upcoming CIN treatment.

9. Results

9.1 Cancer incidence

9.1.1 Cervical cancer incidence

Altogether 22 cases of cervical cancer were observed among the 7 564 women treated for CIN, SIR 2.8, 95% CI 1.7–4.2, compared to the general population. Eleven cases were diagnosed 0.5 to 9 years after treatment, SIR 2.7 (CI 1.4–4.8), ten after 10 to 19 years, SIR 3.1 (CI 1.5–5.7), and one after 20 years, SIR 1.4 (CI 0.04–8.0). CIN 1 and CIN 2 lesions were associated with the highest risk for developing into invasive cervical cancer, SIRs 3.1 (CI 1.4–6.2) and 3.7 (CI 0.8–10.9), respectively (Table 6).

When CKC was set as the reference method, the HRs for cervical cancer incidence were 0.37 (CI 0.07–1.86) for laser, 0.44 (CI 0.09–2.23) for cryo, and 0.32 (CI 0.06–1.57) for LEEP. In comparing all colposcopically guided methods (cryo, laser, and LEEP together) against CKC, the HR for cervical cancer-free survival was 0.37 (CI 0.12–1.18). No clear differences were evident between different grades of CIN in cervical cancer-free survival. Adjusted by histology and method of treatment, the HR for age at first visit in terms of cervical cancer-free survival was 1.37 (CI 1.01–1.86).

Table 6 Cervical cancer incidence after treatment of CIN; overall, and according to the length of follow-up and grade of histology.

	Observed	Expected	SIR	95 % CI
Overall	22	8.0	2.8	1.7–4.2
0.5–9 years	11	4.1	2.7	1.4–4.8
10–19 years	10	3.2	3.1	1.5–5.7
20 + years	1	0.7	1.4	0.04–8.0
CIN 1	8	2.6	3.1	1.4–6.2
CIN 2	3	0.8	3.7	0.8–10.9
CIN 3	3	1.4	2.2	0.5–6.4
CIN NOS	8	3.3	2.5	1.1–4.9

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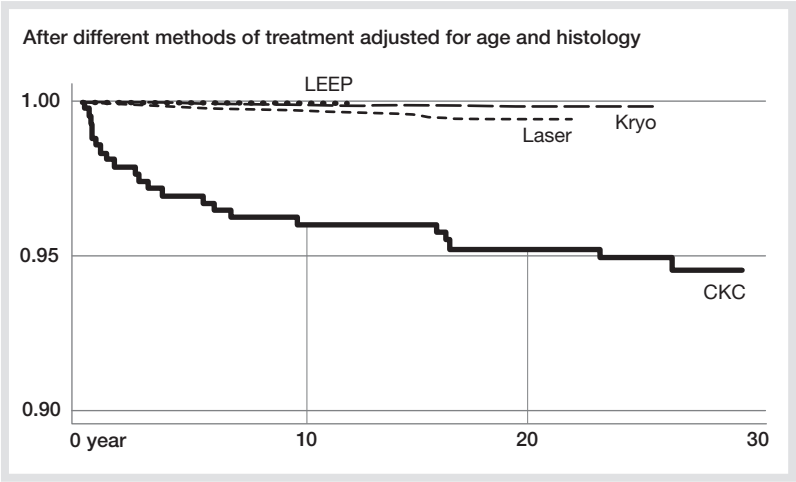
9.1.2 CIN 3 / CIN 3+ incidence

Our material comprised 79 CIN 3+ cases. In CIN 3– or CIN 3+ (CIN 3 or invasive cervical squamous cell cancer, ICC) -free survival, a clear trend emerged toward CKC being the least effective method of treatment. The hazard ratios for CIN 3, when CKC was set as the reference method of treatment, were 0.21 (CI 0.06–0.74) for laser, 0.55 (CI 0.21–1.47) for cryo, and 0.30 (CI 0.12–0.77) for LEEP. The hazard ratios for CIN 3+ when CKC was set as the reference method of treatment were 0.25 (CI 0.09–0.67) for laser, 0.50 (CI 0.22–1.15) for cryo, and 0.27 (CI 0.12–0.62) for LEEP.

When colposcopically guided methods together (cryo, laser, and LEEP) were compared to CKC, the HR for CIN 3 with CKC as the reference method of treatment, was 0.33 (CI 0.16–0.71), and for CIN 3 +, 0.32 (CI 0.17–0.61).

The CIN 3+ free survival after CKC started to differ from other methods of treatment immediately, and continued to be such throughout the follow-up time of the study. No such effect was observed after the other methods of treatment.

Figure 4 CIN 3+ free survival by method of treatment



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9.1.3 HPV-related cancer incidence

After treatment of CIN, significantly increased risks were observable for cancer of the vulva, SIR 4.1 (CI 1.5–8.9), vagina, 12.0 (CI 3.9–28.0), and anus, 5.7 (CI 1.2–17.0).

Compared to CKC, the HRs for HPV-related cancer (cancers of vulva, vagina, and cervix) incidence were 1.02 (CI 0.33–3.12) for laser, 0.49 (CI 0.09–2.38) for cryo, and 0.31 (CI 0.07–1.38) for LEEP, with no clear differences in cervical-, vaginal- or vulvar cancer-free survival by histopathological grade of the CIN lesions either. The hazard ratio of age at treatment, adjusted for histology and method of treatment, in terms of HPV-related cancer-free survival (cervix, vagina, vulva) was 1.43 (CI 1.11–1.85).

All colposcopically guided methods together (cryo, laser, and LEEP) were compared to CKC in cervical-, vulva-, and vaginal cancer-free survival, HR 0.60 (CI 0.22–1.62).

9.1.4 Smoking-related cancer incidence

The incidence of lung or tracheal cancer was elevated compared to that of the general population after treatment of CIN, SIR 2.5 (CI 1.9–3.5). We found a strong correlation between increased risk for lung cancer and long-time follow-up. Age at first visit adjusted by histology and method of treatment was a statistically significant variable for lung cancer incidence in the cancer-free survival analysis, HR 1.94 (CI 1.53–2.45). No difference emerged in lung cancer incidence between different grades of CIN.

Increased risk was also observed for other smoking-related cancers among women treated for CIN compared to the general population, SIR 1.7 (CI 1.3–2.3).

9.1.5. Overall cancer incidence

We identified 448 cases of any cancer among the 7 564 women in Study I treated for cervical intraepithelial neoplasia — 96 more cases than expected, SIR 1.3 (CI 1.2–1.4). The standardized incidence ratios of overall cancer — the incidence of all cancers combined — increased linearly with time.

Altogether 396 cases of any invasive cancer were found after treatment of CIN among 7 466 women in the internal analysis (Study II) with no statistically significant difference in overall cancer incidence between CKC and all three colposcopically guided methods of treatment.

Age at time of CIN treatment adjusted by histology and method of treatment was a statistically significant variable in our cancer-free survival analysis, HR 1.71 (CI 1.60–1.84). No differences appeared in overall cancer incidence between different grades of histology.

9.2 Cancer Mortality

9.2.1 Cervical cancer mortality

Among the 7 104 women treated for CIN who entered the mortality analysis, only three deaths from cervical cancer emerged. Cervical cancer mortality among CIN-treated women was not significantly increased, HR 1.5 (CI 0.4–5.5), and 1.0 (CI 0.3–4.0) after adjustment for socio-economic status (Table 7).

9.2.2 HPV-related cancer mortality

HPV-related gynecological cancer (cancers of cervix, vulva, vagina, or anus) mortality among treated women compared to control population mortality was, even though still based on small numbers (altogether 19 cases), significantly increased, HRs 2.9 (CI 1.1–7.5), and 3.1 (CI 1.1–8.6) after adjustment for socio-economic status (Table 7).

9.2.3 Smoking-related cancer mortality

Mortality from lung cancer was increased both before and after adjustment for socio-economic status, respectively HRs 2.8 (CI 1.8–4.2) and 2.7 (CI 1.8–4.1), and was constantly increased throughout the follow-up. Mortality from the other smoking-related cancers was not elevated above that of the reference cohort, however.

9.2.4 Any cancer mortality

Overall cancer mortality among the CIN-treated was significantly increased before and after adjustment for socio-economic status, HRs 1.4 (CI 1.2–1.7) and 1.4 (CI 1.2–1.7), respectively (Table 7).

9.2.5 Overall mortality

Overall follow-up of mortality was 628 017 woman-years, on average 14.8 years / woman. Overall mortality was 20% higher among treated women, HR 1.2 (CI 1.1–1.3), and 1.1 (CI 1.0–1.3) after adjustment for socio-economic status (Table 7).

Among women less than 40 years old at the time of treatment the overall mortality was significantly higher in the treated cohort than among the reference population, but those over 40 at treatment showed no statistically significant difference in overall mortality between groups.

No statistically significant difference appeared in overall cancer incidence or overall survival between CKC and all three colposcopically guided methods of treatment. With CIN 1 as the reference, the HRs for overall survival were 0.91 (CI 0.60–1.37) for CIN 2, 0.94 (CI 0.72–1.23) for CIN 3, and 0.95 (CI 0.66–1.37) for CIN NOS.

Table 7 Numbers of deaths among treated women versus the reference population. *Cancers of the uterine cervix, vulva, vagina, and anus

	Deaths after treatment for CIN	Deaths, reference population	HR and 95 % CI	HR and 95 % CI adjusted for socioeconomical status
Cervical cancer	3	9	1.5 (0.4–5.5)	1.0 (0.3–4.0)
HPV-related anogenital cancer*	8	11	2.9 (1.1–7.5)	3.1 (1.1–8.6)
Lung cancer	37	72	2.8 (1.8–4.2)	2.7 (1.8–4.1)
All cancers	195	686	1.4 (1.2–1.7)	1.4 (1.2–1.7)
Overall mortality	530	2 251	1.2 (1.1–1.3)	1.1 (1.0–1.3)

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9.3 Fertility and Pregnancy outcome

9.3.1 Fertility (Table 8)

Overall, 2 578 women treated for CIN and 11 642 women in the control population became pregnant during 258 098 woman-years of follow up.

The incidence of any type of pregnancy after treatment (fertility) among women treated for CIN was 20% higher than that for the control population for the first post-treatment pregnancy; adjusted HR 1.20 (p<0.001). The incidence of any pregnancy among treated women with no children before treatment was significantly elevated, adjusted HR 1.29 (CI 1.22–1.39), but among treated women with one or more children prior to treatment it was not; HR 1.09 (CI 0.99–1.20).

The HR for any pregnancy after treatment for CIN (not only the first pregnancy) among women treated for CIN compared to the reference population was 1.15 (p<0.001). Before treatment, the fertility of CIN patients was also elevated; HR 1.06 (p<0.001).

No elevation in hazard ratio of any endpoint occurred immediately after the treatment for CIN.

Table 8 Numbers of women with at least one post-treatment pregnancy, and overall fertility after and before the treatment of CIN.

	Number of treated women pregnant	Number of reference women pregnant	Adjusted HR*	95 % CI	p
First pregnancy after treatment	2 758	11 642	1.20	1.15–1.26	< 0.001
Overall fertility after treatment	4 972	21 363	1.15	1.11–1.18	< 0.001
Overall fertility before treatment	6 942	32 021	1.06	1.04–1.09	< 0.001

*adjusted for number of any pregnancies (0, 1, 2, 3+) and children (0, 1, 2, 3+) before treatment for CIN. Fertility means incidence of any type of pregnancy.

9.3.2 Deliveries (Table 9)

Incidence of deliveries in the first post-treatment pregnancies among the treated women was significantly higher than for the control population; adjusted HR 1.12 (p<0.001). For treated women with no children prior to treatment, the adjusted HR was 1.22 (CI 1.14–1.31), and for women already with one or more children, 0.92 (CI 0.82–1.03).

The incidence of at least one delivery after the CIN treatment among

the treated women compared to that in the reference population was significantly elevated: adjusted HR 1.10 (p<0.001). For women with no deliveries before treatment, the adjusted HR was 1.22 (p<0.001) and for women with at least one delivery before the treatment, 0.91 (p=0.08).

The adjusted HR among women treated for CIN compared to the reference population for delivery at any time after the treatment of CIN was 1.07 (CI 1.03–1.11), higher than the incidence of deliveries before the CIN treatment: adjusted HR 0.99 (CI 0.96–1.02).

Table 9 Number of deliveries in the first post-treatment pregnancy, number of women with at least one delivery after the treatment, and overall numbers of deliveries after and before the treatment of CIN.

	Deliveries among treated for CIN	Deliveries, reference population	Adjusted HR*	95 % CI	p
First pregnancy after treatment	1 932	9 424	1.12	1.06–1.18	< 0.001
At least one delivery after treatment	2 206	10 382	1.10	1.05–1.16	< 0.001
Overall number of deliveries after treatment	3 760	17 414	1.07	1.03–1.11	< 0.001
Overall number of deliveries before treatment	5 200	25 700	0.99	0.96–1.02	0.53

*adjusted for number of any pregnancies (0, 1, 2, 3+) and children (0, 1, 2, 3+) before treatment for CIN, and whether the women had already delivered before treatment.

9.3.3. Spontaneous abortions (Table 10)

The adjusted HR for spontaneous abortions in the first post-treatment pregnancy was 1.04 (p=0.75) between the treated women and the reference population. The incidence of at least one spontaneous abortion was also slightly, but not significantly, higher among women treated for CIN: adjusted HR 1.11 (p=0.10).

The adjusted HR for spontaneous abortion in all post-treatment pregnancies was 1.08 (p=0.35). Spontaneous abortions were slightly elevated among the CIN patients before the treatment date, with adjusted HR 1.16 (p=0.07).

Table 10 Spontaneous abortions in the first post-treatment pregnancy, number of women with at least one spontaneous abortion, and overall numbers of spontaneous abortions after and before the treatment of CIN.

	Spontaneous abortions among those treated for CIN	Spontaneous abortions, reference population	Adjusted HR*	95 % CI	p
First pregnancy after treatment	80	412	1.04	0.80–1.36	0.75
At least one spontaneous abortion after treatment	159	707	1.11	0.94–1.33	0.23
Spontaneous abortions after treatment	171	770	1.08	0.92–1.28	0.35
Spontaneous abortions before treatment	193	816	1.16	0.99–1.36	0.07

*adjusted for the number of any pregnancies (0, 1, 2, 3+) and children (0, 1, 2, 3+) before the treatment for CIN, and whether the women had had a spontaneous abortion before the treatment or not.

9.3.4 Extra-uterine pregnancies (Table 11)

The adjusted HR for extra-uterine pregnancy in the first post-treatment pregnancy was 1.93 ($p<0.001$). For women with no children prior to the treatment and for women with at least one child before the treatment, the adjusted HRs were in the first pregnancy 2.22 ($p=0.003$) and 1.47 ($p=0.30$), respectively. The incidence of at least one post-treatment extra-uterine pregnancy was also elevated among the treated: adjusted HR 1.81 ($p= 0.003$).

Overall adjusted HR for extra-uterine pregnancy was 1.88 (CI 1.50–2.36) after treatment and 1.40 (1.13–1.74) before the treatment, with no significant difference.

9.3.5 Molar pregnancies

We observed 146 molar pregnancies — 26 among the treated and 120 in the reference population — in the first post-treatment pregnancy, and 295

Table 11 Extra-uterine pregnancies in the first post-treatment pregnancy, number of women with at least one induced extra-uterine pregnancy, and overall numbers of extra-uterine pregnancies after and before the treatment of CIN.

	Extra-uterine pregnancies among those treated for CIN	Extra-uterine pregnancies, reference population	Adjusted HR*	95 % CI	p
First pregnancy after treatment	59	151	1.93	1.35–2.76	< 0.001
At least one extra-uterine pregnancy after treatment	89	229	1.81	1.40–2.34	< 0.001
Extra-uterine pregnancies after treatment	107	267	1.88	1.50–2.36	0.005
Extra-uterine pregnancies before treatment	105	369	1.40	1.13–1.74	0.0002

*adjusted for number of any pregnancies (0, 1, 2, 3+) and children (0, 1, 2, 3+) before treatment for CIN, and whether the women had had an extra-uterine pregnancy before treatment.

molar pregnancies overall after CIN treatment. The adjusted HR for molar pregnancy in the first post-treatment pregnancy was 1.04 (CI 0.80–1.36). The incidence of at least one molar pregnancy was slightly higher among women treated for CIN: adjusted HR 1.11 (CI 0.81–1.52).

9.3.6 Induced abortions (Table 12)

The incidence of induced abortions in the first post-treatment pregnancy among the treated women was significantly elevated, adjusted HR 1.62 ($p<0.001$). Hazard ratios for women with no children was 1.65 (CI 1.40–1.94) and at least one child 1.83 (CI 1.49–2.24).

The incidence of at least one induced abortion was also elevated among the treated, the adjusted HR being 1.53 ($p<0.001$). The adjusted hazard ratios for at least one post-treatment induced abortion for women with no induced abortion and at least one induced abortion before the treatment were 1.70 ($p<0.001$) and 1.09 ($p=0.58$), respectively. The overall

HR for all induced abortions after CIN treatment was 1.54 (CI 1.43–1.66). Before the treatment date, induced abortions were already more common among those treated, HR 1.39 (CI 1.31–1.47), with no significant difference from the incidence observed after treatment.

Table 12 Number of induced abortions in the first post-treatment pregnancy, number of women with at least one induced abortion, and overall numbers of induced abortions after and before the treatment of CIN.

	Induced abortions among those treated for CIN	Induced abortions, reference population	Adjusted HR*	95 % CI	p
First pregnancy after treatment	481	1 535	1.62	1.45–1.82	< 0.001
At least one induced abortion after treatment	695	2 245	1.53	1.40–1.67	< 0.001
Overall number of induced abortions after treatment	881	2 670	1.54	1.43–1.66	< 0.001
Overall number of induced abortions before treatment	1 406	4 978	1.39	1.31–1.47	< 0.001

*adjusted for number of any pregnancies (0, 1, 2, 3+) and children (0, 1, 2, 3+) before treatment for CIN, and whether the women had had an induced abortion before treatment.

10 Discussion

10.1 Comparison of the results to those of other studies

10.1.1 Cervical cancer incidence

We observed in Study I that cervical cancer incidence was 2.8-fold higher among the treated than in the general population — about 22/100 000 woman-years. Soutter et al (1997) concluded the incidence of cervical cancer after treatment of CIN to be 5.8/1 000 women or 85/100 000 woman-years, and this incidence remained elevated for at least 8 years. In that study, the follow-up was performed via cytological samples, with no systematic follow-up after the treatment. This might have resulted in women with symptoms attending follow-up more frequently and therefore would explain the slightly higher incidence. Furthermore, the results of more recent studies are very much in line with our findings: The incidence of cervical cancer after CIN treatment was about 2.8-fold higher than expected (Soutter et al 2006); SIR 2.3 compared to that of the general population after treatment of CIN 3 (Strander et al 2007); SIR 1.7 compared to a general population (Jakobsson et al 2010); and cervical cancer incidence after CIN treatment 37/100 000 woman-years (Melnikow et al 2009). The results in the studies including only cervical carcinoma in situ lesions did not differ from ours: SIR 2.3 to 3.4 compared to the general population figure after treatment of carcinoma in situ (Hemminki et al

2000a, Levi et al 1996); and 50/100 000 (Pettersson and Malker 1989).

When we studied the differences between the treatment methods and set CKC as the reference method, the hazard ratios for cervical cancer incidence were 0.37 for laser, 0.44 for cryo, and 0.32 for LEEP. In comparing all colposcopically guided methods (cryo, laser, and LEEP together) to CKC, cervical cancer-free survival was 0.37 (95% CI 0.12–1.18). We observed a trend towards cryo's being the worst of the three colposcopically assisted methods in terms of CIN 3+ incidence as well, but we lacked cases of incident cancers and therefore statistical power. In terms of CIN 3+ incidence in our study, CKC was clearly the worst method. Nor did another Finnish study published recently find any significant differences in cervical cancer incidence between the different treatment modalities (Jakobsson et al 2010). Melnikow et al (2009) found cryo to be significantly the worst in terms of cervical cancer incidence, OR 2.98, compared to other methods of treatment. That data included no women treated with CKC.

For different grades of CIN, Melnikow et al (2009) observed cervical cancer incidence to be increased to a greater extent after CIN 3 than after CIN 1 (OR 4.1), and for age over vs. under 40 at the time of CIN treatment diagnosis, the OR was 1.75. A clear difference was that we found no variation in CIN 3+ or cervical cancer incidences between different grades of CIN. This may be due to different classification of the lesions: We used the first diagnostic biopsy to categorize patients; for instance the lesions first treated as CIN 1 were called CIN 1 lesions throughout the follow-up in our data. The Melnikow group decided that the worst histological diagnosis in their analysis to appear within 6-month follow-up after the initial visit to be the index histology. This is evident in the distribution of CIN 1 cases in the two data sets: 19% of all CIN cases as CIN 1 cases for the Melnikow group, vs. 32% CIN 1 cases of all the CIN cases in our own data. This difference in proportion of CIN grades, due to differing classifications, might well explain this difference. Increasing age at treatment of CIN significantly raised our cancer incidence, to HR 1.4, a finding in line with the Melnikow group's.

Women treated for CIN of any grade by any treatment method are at increased risk for cervical cancer, compared to the risk of the standard population, for at least 20 years after the treatment. This suggests that women diagnosed with CIN 1 and 2 possess the same risk factors as do women with CIN 3, and therefore are equally at risk for invasive cancer. Still, screening

for and treating cervical preinvasive lesions reduces the burden of invasive cancer significantly: We estimated the effect of CIN treatment in our data based on an earlier study of progression rates of CIN 3 lesions in Finland (Hakama and Räsänen-Virtanen 1976) that suggested 28 to 39% of cases of CIN 3, when left without treatment, would progress to invasive cancer. Another study, following women with cervical carcinoma in situ, reported that when left untreated, about 22% of the lesions would progress to invasive cervical cancer (McIndoe et al 1984). We had 837 cases of CIN 3 lesions among women aged 30 to 59. Based on the Finnish estimate, 234 to 326 cases would have developed into invasive cervical cancer, whereas we observed only three. The treatment effect therefore may have approached 99%.

10.1.2 HPV-related cancer incidence

After treatment of CIN, we observed increased risks for cancer of the vulva (4.1, 95% CI 1.5–8.9), vagina (12.0, CI 3.9–28.0), and anus (5.7, CI 1.2–17.0). Few studies of other HPV-related cancer incidences after treatment of CIN were retrievable, and their results were very similar to ours. The point estimates between the studies vary slightly, but women treated for CIN clearly are at increased risk for cancers of the vagina, vulva, and anus.

A later Finnish study found somewhat similar increases in cancers at these primary sites (SIR 6.15 for cancer of the vulva, 9.08 for vaginal cancer, and 3.56 for anal cancer) (Jakobsson et al 2010). A Swedish Cancer Registry-based study of women treated for CIN 3 published after ours found increased risk for cancers of the vagina (incidence relative risk, (IRR), 6.74, 95% CI 5.24–8.56), vulva (2.22, CI 1.79–2.73), and anus (4.68, CI 3.87–5.62) compared to risk in the general population (Edgren and Sparen 2007). An earlier study using the same database found cancers of the anus and female genitalia (including vagina, vulva, and unspecified cancer of uterus) to be increased similarly after treatment of cervical carcinoma in situ (Hemminki et al 2000a). Evans et al (2003) documented, as well, an increase in incidence of cancers of the anus (SIR 5.9), vulva (SIR 4.4), and vagina (SIR 18.5) after treatment of CIN 3.

10.1.3 Smoking-related cancer incidence

Our study showed, after treatment of CIN, incidence of cancer of the lung or trachea compared to the general-population incidence was elevated (SIR 2.5, 95% CI 1.9–3.5). An increased risk was also observable for other smoking-related cancers (cancers of larynx, tongue, mouth, other larynx besides naso- or hypopharynx, pancreas, bladder and kidney) among women treated for CIN compared to the general population (SIR 1.7, CI 1.3–2.3). A later Finnish study found a similar increase in incidence of smoking-related cancers (SIR 1.45 CI 1.12–1.86) (Jakobsson et al 2010). A study based on records of the Swedish Cancer Registry observed that after treatment of cervical carcinoma in situ, cancers of the lung (SIR 2.17) and urinary bladder (SIR 1.4) were increased (Hemminki et al 2000a). After treatment of cervical carcinoma in situ, SIR 2.2, cancers of the lung, mouth or pharynx, esophagus, and urinary bladder were more common than in the general population, in another study about treated cervical carcinoma in situ lesions (Levi et al 1996). Evans et al (2003) similarly found that incidence of cancers of the lung (SIR 1.5) and kidney (SIR 1.6) was increased after treatment of CIN 3 (Evans et al 2003).

All these results are from studies performed methodologically the same way as ours: comparison of a treated cohort with the incidence in the general population. Even though we also included women treated for CIN 1 and 2 lesions, the results obtained by other groups are very much in concordance with our observations. Women with CIN of any grade are documented to smoke more often than does the general population. Although smoking is associated with increased risk for cervical cancer, its risk for developing other cancers is significant as well.

10.1.4 Mortality

To our knowledge, few studies about mortality after treatment of CIN exist. Due to the excellent registers in Finland, two of them were performed here as well. We observed no increase in cervical cancer mortality among treated women; HR 1.5, (CI 0.4–5.5), and 1.0, (CI 0.3–4.0) after adjusting for socio-economic status. In another Finnish study by Jakobsson et al

(2009a), the standardized mortality ratio (SMR) for cervical cancer was 7.7 (CI 4.23–11.15). No information on socio-economic risk factors was included. No linkage between the CIN 3 cases gathered from THL and the Finnish Cancer Registry was performed by the Jakobsson group, leaving prevalent or earlier invasive cervical cancer cases in the data. This may at least partly explain our difference in cervical cancer mortality. When they later excluded cervical cancer cases from the data, their data still showed, for cervical cancer mortality, an increase (Personal communication with M Jakobsson).

Mortality, SMR, from all diseases and medical conditions in the study by Jakobsson et al (2009a) was 1.13, 95% CI 1.01–1.26, from all cancers 1.09 (CI 0.91–1.27), and from injury deaths 1.31 (CI 1.03–1.58). All-disease mortality, and mortality from accidents were practically identical in our results, as well. In these categories, in both studies, the cause of death came mainly from the Causes of Deaths Registry. Mortality from any cancer was somewhat lower than in our study 1.1 (0.9–1.3) vs. 1.4 (1.2–1.7). They had altogether 145 cancer deaths among almost 26 000 women; we, on the other hand, had 195 cancer deaths among 7 100 women, indicating a possible difference in duration of follow-up. The Jakobsson group paper lacks total length of follow-up in woman-years. In our data, all cancer deaths were further verified from the Finnish Cancer Registry, and cancer deaths additional to those received from the Cause of Death Register did in fact emerge. Our study was based on treatments in one hospital, Helsinki University Central Hospital. The study population of the Jakobsson group comprised women gathered from a nationwide registry. Perhaps these differences in design, follow-up time, and nationwide variation in the efficacy of the treatment and follow-up explain the differences between these results.

A study by Hakama et al (2004), reported excess mortality among women with cervical carcinoma in situ. Their risk of death (>10%) was substantially increased only at advanced ages and was independent of age at diagnosis. Our own difference in overall mortality between treated women and our reference cohort had a tendency to diminish, the older were the women when treated, but tended to increase between the two cohorts the longer was the follow-up time, thus supporting these earlier findings.

Despite effective treatment and follow-up, the mortality from other than cervical, and other than HPV-related cancers as well, is elevated among the women treated for CIN. Smoking, in particular, is associated with both cervical cancer and lung cancer, and women treated for CIN here had significantly increased lung cancer mortality. Women with any grade of CIN have other risk factors, in addition to HPV, which cause significant morbidity and mortality; this should be taken into account when both evaluating and planning cervical cancer-preventive activity.

10.1.5 Fertility and pregnancy outcome

Earlier studies about fertility have not reported a significant reduction in fertility, or fertility has been higher in the treated cohort. One review of small, older studies concluded that no impairment in fertility had been observed after treatment of CIN (Hammond and Edmonds 1990). Based on a postal questionnaire of 250 women treated with LEEP, again no reduction in fertility was observed: This was during a 3-year follow-up (Bigrigg et al 1994). Another cohort study comparing pregnancy outcomes of 433 women before and after treatment of CIN with laser conization concluded that the treated women actually had more pregnancies (277 vs. 177, $p<0.01$); the mean duration of that study interval was 3.8 years (Spitzer et al 1995.) In a cohort of 574 women treated with LEEP, the incidence of pregnancies was again slightly higher among the treated than in the general population (8.5/100 women vs. 7.4/100 women) (Ferency et al 1995).

In our cohort of 36 000 women, for whom average duration of follow-up was almost 15 years, we calculated the incidence of at least one pregnancy after the treatment, the incidence of all pregnancies before and after treatment, and the incidence of pregnancies in increasingly long follow-up periods after treatment. As all these outcomes were significantly more common among the treated than in the reference population, we can assume that the treatment causes neither reduction in fertility right afterwards nor any infertility at all. The point estimates were actually higher after than before treatment, a finding in line with previous findings.

The incidence of deliveries among the treated women in the first post-treatment pregnancy and in all post-treatment pregnancies was significantly increased, and more often than did women in the reference population, the treated women had at least one delivery. Our incidence of deliveries before the treatment was not increased. A study based on a postal questionnaire involving 653 women treated for CIN reported no difference in incidence of deliveries between the treated and a healthy reference cohort (Cruickshank et al 1995). Another study comparing the pregnancies of 433 women before and after treatment of CIN with laser conization observed a significantly higher incidence of deliveries after the treatment, 163 vs. 112 ($p<0.01$) (Spitzer et al 1995). This elevation in the post-treatment deliveries here and in the study of Spitzer et al may be due to the psychosocial effect of the treatment: Although it is documented that after the treatment for CIN women experience anxiety, distress and low self-esteem, and spontaneous interest in sex and frequency of intercourses have been reduced (McDonald et al 1989, Marteau et al 1990, Le et al 2006, Hellsten et al 2008), we observed no corresponding decrease in fertility. The treatment might have actually served as a "wake up call": Despite the reduction in spontaneous interest in sex and in frequency of intercourse for a while after treatment, the urge to become a mother might have increased simultaneously. This is supported by findings that the post-treatment incidence of deliveries was significantly increased only among the women with no children prior to treatment. Psychosocial effects caused by the treatment are, in our opinion, the most likely explanation behind the observed increase in the incidence of deliveries.

We observed no increase in incidence of spontaneous abortion among the treated women, with point estimates actually lower after than before treatment. Especially among women with at least one child before treatment, we observed no significant difference in spontaneous abortion incidence. Nor did previous small studies find any increase in spontaneous abortion rates (Weber and Obel 1979, Tan et al 2004). Older studies, again based on small numbers of both women and endpoints, did find some increase in first and second trimester spontaneous abortions after CKC (Lee 1978, Jones et al 1979). We saw no such effect even after CKC. A Norwegian registry-based study, however, observed a three-fold increase in late spontaneous abortion incidence after any treatment of any grade

of CIN (Albrechtsen et al 2008). As we lacked knowledge of the exact pregnancy weeks in the spontaneous abortion data, our results are not directly comparable to those of Albrechtsen et al.

Only one previous study of extra-uterine pregnancy rates exists: For 433 women treated with laser conization, their pregnancies were studied before and after treatment. No comparison to the general population was made, and no observation of any increase in incidence of extra-uterine pregnancies after treatment (Spitzer et al 1995). We observed an elevated incidence of extra-uterine pregnancies after treatment of CIN, both in the first post-treatment pregnancy and overall, and the treated women had at least one extra-uterine pregnancy more often as well. The incidence of ectopic pregnancies was already elevated before treatment, however, and we observed no significant increase in incidence right after the treatment, a result in line with Spitzer et al (1995). Previous extra-uterine pregnancy is a major risk factor for another ectopic pregnancy, so our results were adjusted for this event before treatment. Genital infections, especially Chlamydia trachomatis, is a major cause of pelvic inflammatory disease (PID), and women with CIN are well documented to have a higher incidence of this infection than in the general population. Based on all of this, we can assume that other risk factors for PID and tubal damage that these CIN patients possess in excess when compared to the general population rather than the CIN treatment itself causes their higher post-treatment incidence of extra-uterine pregnancies.

The incidence of induced abortions was increased among those treated, both before and after treatment of CIN. The treated women had a higher incidence of at least one induced abortion as well. Some evidence exists for an increased induced-abortion rate and cervical cancer or cervical dysplasia between the treated and a healthy population or occurring after than before treatment in the same cohort (Parazzini et al 1989, Wang and Lin 1995, Spitzer et al 1995). We observed a slightly higher, but not a statistically significant, incidence of post-treatment induced abortions, but no evidence whatsoever about the treatment per se as significantly elevating induced abortion incidence. The anxiety and distress caused by treatment might perhaps be expected to result for some individuals in an increased willingness to terminate pregnancies.

10.2 Strengths and limitations

The follow-up in all four studies was based on nationwide registers: Cancer cases were retrieved from the Finnish Cancer Registry, causes of deaths were from Statistics Finland's Cause of Death Register; dates of death, of emigration, and birthdays of children came from the Finnish Population Registry, and data on the endpoints concerning reproductive health, i.e. dates of induced and spontaneous abortions, extra-uterine pregnancies, molar pregnancies, and sterilizations from the THL (National Institute for Health and Welfare) Care Registers for Social Welfare and Health (previously called STAKES Hospital Discharge Register). The socioeconomic status of the women for Study III was retrieved from Statistics Finland's census records between 1970 and 2004 (Sosioekonomisen aseman luokitus 1989). The records in all these organizations are considered reliable for epidemiologic research (Keskimäki and Aro 1991, Teppo et al 1994, Gissler and Shelley 2002, Gissler and Haukka 2004), and the linkage procedures were precise and trustworthy in the current study.

The number of women treated for CIN, the number of most endpoints, and the follow-up time in all four studies were superior to those in earlier studies. Earlier data showed, for instance, cervical cancer incidence to be increased after CIN treatment for 8 years (Soutter et al 1997), and on average we had a follow-up of 12 years per woman in Study I.

The CIN cases were collected manually from paper- and microfilm archives for women treated for CIN as well as by a computerized database search of hospital records of Helsinki University Central Hospital, Department of Obstetrics and Gynecology. Possible flaws in personal identifiers and other data in the registers used might predispose to bias. All personal identifiers were linked with the Finnish Population Registry to assure the correctness of the PIDs. Due to a wrong PID in the hospital records, a few women might actually have never been treated for CIN. This source of systematic bias can be considered minor and not affecting results significantly.

The Finnish Cancer Registry has records of cervical carcinoma in situ cases from 1954 onwards and for CIN 3 cases from the mid 1990's (Finnish Cancer Registry 2009). No nationwide quality-assured register of CIN 1 and 2 cases exists for the whole follow-up time in our study. It is

thus possible that some women have actually been treated for CIN before appearing in the hospital records studied, and hence in our data. CIN is also a dynamic lesion: Most CIN 3 lesions obviously developed with time through CIN 1 and 2, and women who were diagnosed first with CIN 1 and later with cervical cancer might have, in between, been treated for CIN 2 and CIN 3 as well. The diagnosis gathered from the hospital records might therefore have changed toward the worse or the better later on without our knowledge, and verification or information bias might thus arise, possibly affecting our results. We can still assume that the overall distribution of grades of CIN would remain the same. Furthermore, all women treated for CIN 1, 2, 3, and CIN NOS, share the same risk factors: HPV infection, smoking, early sexual debut, multiple sexual partners, and all women in our data were by definition diagnosed and treated for CIN. It is also possible that the initial diagnosis in hospital records was CIN, even though it is actually a question of an already invasive cancer, not revealed until later in the diagnostic process. In this case, the diagnosis of cancer may not have been transferred into the hospital records. Because of this possible systematic bias in the records, we chose to use a 6-month lag-period in every analysis, i.e. cervical cancer diagnosis was impossible until after 6 months of follow-up. All women with cervical cancer diagnosed within 6 months of treatment were excluded from the follow-up, or the follow-up of all endpoints started 6 months after treatment, depending on the study in question.

In Study I, the comparison in cancer incidence was between the cancer incidence of the treated women and the cancer incidence of the general population in the Helsinki-Uusimaa region. In Studies III and IV, comparisons were between the treated women and their matched reference cohort. That other CIN treatments among the women in both reference groups also existed was a disadvantage with some effect on our results. Treated women in the reference group diminish the difference in risk factors between the groups and therefore are more likely to slightly dilute than to exaggerate our results.

As endpoints, cancer and death almost always are recorded in these registers, and no source of possible follow-up bias from missing data should therefore be expected in Studies I to III. On the other hand, women treated for CIN have most likely participated in intensified follow-up, with

several extra colposcopies done after the treatment. Diagnosis of both cervical cancer and cancers of the vagina, vulva, and anus are therefore more likely to appear earlier among these women, affecting our results. In Study IV, diagnosis of extra-uterine pregnancy or spontaneous abortion might be somewhat susceptible to follow-up bias: Women less conscious of their health might not visit a physician for abnormal vaginal bleeding and therefore do not appear in these THL registers. This kind of behavior may be more common among the women with CIN. On the other hand, women treated for CIN in our data have participated in nationwide or spontaneous screening, and attended colposcopy. These women thus cannot definitely be assumed not to react to vaginal bleeding at a lower rate than would healthy women in the reference populations, meaning no significant follow-up bias is likely to arise here, either.

The major confounding factor in cancer incidence, mortality, and reproductive health is age. In Studies I and II, all models were adjusted for age. In Studies III and IV, as the reference population we used women matched by age and municipality of residence at the time of CIN treatment. If information in the registers was limited, these limitations were similar for both cohorts and therefore do not compromise results. On the other hand, the treated women in our cohort were mainly of screening age at the time of treatment, under 60 (Table 1). We might thus be partially missing, in both cancer incidence and mortality analyses, those older women who, after cessation of screening, develop invasive cervical cancer.

In study of the treatment effects in depth, especially as to cancer incidence, a major disadvantage was that we lacked systematic information about possible treatment failures and multiple treatments which would have enabled us to differentiate more carefully the cancer risk after different grades of CIN and factors behind its progression to cancer. The classification system in the hospital records prevented us from differentiating between laser ablation and laser conization. This would have provided us the opportunity to analyze excisional and ablational treatments separately.

One of our main goals was to determine cervical cancer mortality among the women treated for CIN. Among them, we observed only three deaths due to cervical cancer. Based on that small number of deaths, the cervical cancer mortality figures between the cohorts lacked statistical

power. Perhaps a retrospective cohort study is not optimal for this kind of rare event. Nevertheless, no increase in cervical cancer mortality was observable, and adjusting for socio-economic status resulted in an HR of 1.0. In Finland, due to nationwide screening, cervical cancer is nowadays a rare disease, and death from cervical cancer is an even less common phenomenon.

One major source of the confounding is lack of information about smoking and sexual behavior in Studies I, II, and IV. These missing data did not cause a marked problem in evaluating the overall effectiveness of CIN treatments, but could have left a certain confounding in our comparison between the treated and reference populations. Socio-economic status correlates with smoking as well as with cervical cancer and CIN incidence, and can serve as a surrogate variable for smoking. Still some residual confounding can remain in the results. Study III had information about socio-economic status, which varied significantly over time, census by census; we used socio-economic status from the census nearest to the day of treatment. This selection of only one social class to use throughout the follow-up, even though common in other studies, might not have been the most informative to estimate the effect of socio-economic status on mortality. Some confounding effect of socio-economic status, and therefore of smoking, might remain in our results. On the other hand, adjusting for socio-economic status in Study III did not change any results significantly. The lack of socio-economic data in other studies can thus be considered not a major disadvantage.

Information about hysterectomies was irretrievable in any of the studies, or about the exact pregnancy week at spontaneous abortion in Study IV. If any bias had occurred due to a larger proportion of hysterectomies among CIN patients, it would have diluted, not exaggerated the results of cancer incidence, mortality, and pregnancy outcome. Differences in reproductive outcomes detected in Study IV can therefore be considered valid. Use of oral contraceptives, or in Study IV overall willingness to get pregnant, might have left some residual confounding in the results. Previous studies about pre-term births and other adverse pregnancy outcomes suggested CIN treatment itself to be the cause of later complications, rather than the risk factors predisposing to treatment and to other morbidity. We tried to take this into account by estimating the hazard ratio both before

and after the treatment; based on the confidence intervals, we were able to conclude that the treatment itself seemed not to raise the incidence of spontaneous abortions, extra-uterine pregnancies or induced abortions.

10.3 Summary and implications

The risk for cervical cancer after treatment of CIN is elevated for at least 20 years compared to that of the general population. In addition to cervical cancer, women treated for CIN are at increased risk for other HPV-related cancers, such as of the vagina, vulva, and anus. Furthermore, risk for lung cancer and other smoking-related cancers was also elevated.

Incidences of cervical cancer and HPV-related gynecological cancers are elevated after all the four types of conservative treatments (CKC, cryotherapy, laser conization or ablation and LEEP), regardless of the grade of CIN treated. CIN 3 or CIN 3+ incidence was significantly elevated after CKC compared to the colposcopically assisted methods of treatment. Even if the effect of treatment is good, the treated women are at increased risk for developing cervical cancer compared to the risk in the general population, especially at older ages. This calls for intensified surveillance and systematic screening likely to last for a lifetime with extra focus on the other primary sites as well.

Cervical cancer is a rare disease in Finland, and cervical cancer mortality was not significantly elevated after treatment of CIN. After adjusting for socio-economic status, the HR for those treated vs. the reference population was 1.0: The treatment is highly effective: The estimated treatment effect of cervical cancer prevention in our data was up to 99%, and only three deaths from cervical cancer were observed among the 7 000 women treated for the precancerous lesion. Excess mortality among these women did, however, occur from other causes, especially from lung and other cancers, necessitating even more attention towards cessation of smoking.

The treatment itself seems neither to elevate the risk for the unwanted reproductive health outcomes studied nor to harm fertility. The treated women are more fertile and deliver more often than does the reference population, also after treatment. The incidence of extra-uterine pregnancies and induced abortions was already elevated before the treatment.

General information about the harmlessness of the treatment in regards to fertility, measures to reduce anxiety experienced by women about the treatment, and especially advisories about the use of contraception, especially condoms, are important interventions to reduce the rate of induced abortions and possibly of ectopic pregnancies, as well as HPV infections.

11 Conclusions

The following conclusions can be drawn:

1. We observed that cervical cancer incidence, HPV-related gynecological cancer incidence, and smoking-related cancer incidence were elevated among women treated for CIN.

2. Neither grade of the CIN lesion nor method of treatment affected the success of CIN treatment in terms of cancer-free survival. In terms of CIN 3 or CIN 3+ free survival, the three colposcopically assisted treatment methods were superior to non-colposcopically assisted CKC. Increasing age at treatment was associated with increasing risk for developing CIN 3 or cervical cancer.

3. After treatment of any CIN, mortality from cervical cancer was not elevated. Instead, mortality from all HPV-associated cancer, all cancers, and overall mortality were significantly increased compared to levels in the reference population.

4. Women treated for CIN became pregnant and delivered more often than did the reference population. In terms of the pregnancy outcomes studied, the CIN treatment itself did not raise

the incidence of induced abortions or of abnormal pregnancies.
The CIN treatment had no negative effect on future fertility.

Altogether, the conservative ablative and excisional treatment methods for CIN are effective in terms of cervical cancer prevention and are safe in terms of the outcomes studied regarding future fertility. As the cancer risk subsequent to treatment is elevated for at least 20 years, the proper follow-up is as important as the treatment itself to catch the inevitable cancer cases sufficiently early to avoid cancer deaths.

CIN can be considered a risk-marker: It is a marker for increased cancer incidence, for increased cancer and overall mortality, and for increased extra uterine pregnancy and induced-abortion incidence. These are not caused only by HPV; smoking, genital tract infections, and perhaps psychosocial factors also play a major role. In the management process for CIN patients, regular condom use and most of all cessation of smoking require encouragement.

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