Tampere University

INDIRA ADHIKARI

Association of Sexual Behaviour and Oral Contraceptive Use with Cervical Neoplasia

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ACADEMIC DISSERTATION
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of Tampere University,
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Tampere University, Faculty of Social Sciences
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Dedication

Dedicated to my dear parents, my husband, and our darling daughter Deeva.

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September 15, 2022

Indira Adhikari

ABSTRACT

Cervical cancer remains the fourth most common cancer in women worldwide. It is associated with high mortality even if much progress has been made in its prevention and treatment. The fact that the necessary cause of cervical cancer is sexually transmitted human papillomavirus (HPV) infection makes it complicated because there are many other sexually associated factors which amplify the risk of HPV infection, such as *Chlamydia trachomatis* infection, a high number of sexual partners and use of hormonal contraceptives instead of condoms. These factors may put young girls and women at reproductive age at risk of pre-cancerous cervical lesions and cancer.

There is ample evidence of how HPV and Chlamydia increase the risk of cervical cancer, whereas the result of research on the use of hormonal/oral contraceptives is mostly contrasting. The reasons for this could also be due to the duration of contraceptive use in addition to the contraceptive's hormonal composition and the relative decrease of condom use.

The general aim of this thesis was to find out what role does use of oral contraceptives (OCs) play in cervical neoplastic changes. The required information was taken from the PATRICIA trial of HPV16/18 vaccine efficacy, followed up for 4 years, a cluster randomised trial on the effectiveness of different HPV vaccination strategies and Finnish Student Health Service (FSHS) and Finnish Maternity Cohort (FMC) serum bank. Four peer-reviewed studies are included in the dissertation. In Study I, we assessed the risk between persistent HPV infection and duration of oral contraceptive use among the women who visited the Finnish Student Health Service (FSHS). Long-term use of oral contraceptives was associated with HPV seropositivity, a surrogate of persistent HPV infection.

In Study II, we evaluated the risk of cervical atypia in oral contraceptive users among the women participating in the control arm of the PATRICIA trial of HPV16/18 vaccine efficacy. We found no increased risk of cervical atypia in oral contraceptive

users compared to non-users. Instead, the established use of oral contraceptives was protective against cervical atypia compared to non-users of oral contraception.

Study III examined the risk of cervical atypia associated with the changing interval between menarche and the start of sexual activity among the women participating in the control arm of the PATRICIA trial of HPV16/18 vaccine efficacy. A short interval between menarche and the start of sexual activity was not associated with an increased risk of cervical atypia. However, the risk of cervical atypia due to *Chlamydia trachomatis* infection was increased when the interval between menarche and the start of sexual activity was short.

In Study IV, we assessed the risk between squamous intraepithelial lesions (SILs) and the combined effect of *Chlamydia trachomatis* and the duration of oral contraceptive use among the women participating in the community randomised HPV vaccination effectiveness trial in Finland. The risk of squamous intraepithelial lesion was increased in *Chlamydia trachomatis* positive women who were also using oral contraceptives for 5 or more years.

The findings of this thesis emphasise the importance of sexual health counselling, especially contraceptive counselling, among adolescent and young adult women. Infection with either HPV, *Chlamydia trachomatis* or both cannot always be treated and cured at once. Some infections persist depending upon the person's physiological condition and lifestyle factors, such as smoking, multiple sexual partners, parity, use of contraceptive methods and socioeconomic status. The persistence of high-risk HPV infection increases the risk of severe cervical lesions. Even though the progression period of HPV-related cervical cancer is relatively long, the risk is high, especially in those countries where there is no HPV vaccination programme. In this study, we found an increased risk of SILs in women who have *Chlamydia trachomatis* and have used OCs for 5 or more years in an HPV-vaccinated population. Also, high-risk populations with no HPV vaccination will continue to face an increased burden of cervical cancer. Therefore, proper sexual health counselling needs to be promoted. Prospective studies on the use of oral contraceptives and the risk of cervical neoplasia are needed to further elaborate this association.

TIIVISTELMÄ

Kohdunkaulan syöpä on edelleen neljänneksi yleisin naisten syöpä maailmassa. Siihen liittyy korkea kuolleisuus, ennaltaehkäisyssä ja hoidossa tapahtuneesta edistymisestä huolimatta. Se, että ihmisen papilloomavirus (human papillomavirus, HPV) infektio on kohdunkaulansyövän välttämätön syy mutkistaa tilannetta sikäli, että monet muutkin seksin yhteydessä tarttuvat tekijät, kuten Chlamydia trachomatis infektio, korkea seksipartnereiden määrä ja hormonaalinen ehkäisy kondomin käytön sijasta, voivat lisätä HPV infektioon liittyvää riskiä. Nämä tekijät voivat saattaa nuoret tytöt ja hedelmällisessä iässä olevat naiset riskiin kohdunkaulansyövän ja sen esiasteiden suhteen.

On olemassa runsaasti todisteita siitä miten HPV ja klamydia lisäävät kohdunkaulansyövän riskiä, kun taas tulokset hormanaalisesta ehkäisystä/ehkäispillereiden käytöstä ovat tässä suhteessa ristiriitaisia. Syitä tähän voisivat olla vaihtelu ehkäisyn pituudessa ja ehkäisyvalmisteiden hormonisisällössä, sekä kondomin käytön suhteellinen väheneminen.

Tämän väitöskirjan tavoitteena oli löytää vastaus kysymykseen: mikä rooli ehkäisypillereiden käytöllä on kohdunkaulan pahanlaatuisissa muutoksissa? Tarvittavat tiedot saatiin HPV16/18 rokotteen tehoa koskeneesta, 4 vuoden mittaisesta PATRICIA-trialista, eri HPV-rokotusstrategoita koskeneesta paikkakuntasatunnaistetusta trialista, ja Ylioppilaiden Terveydenhoitosäätiön (YTHS) ja Finnish Maternity Cohort (FMC) -seerumipankin aineistoista. Väitöskirjaan sisältyy neljä vertaisarvioitua artikkelia. Tutkimuksessa I määritimme riskin pitkittyneen HPV-infektion ja ehkäisypillereiden käytön pituuden välillä YTHS:n asiakkailla. Pitkäkestoinen ehkäisypillereiden käyttö liittyi HPV vasta-ainepositiivisuuteen, joka on pitkittyneen HPV-infektion merkki.

Tutkimuksessa II arvioimme kohdunkaulan poikkeavien solulöydösten riskiä ehkäispillereiden käyttäjillä PATRICIA-trialin verrokki-armissa. joka ei saanut HPV16/18 rokotetta. Emme havainneet poikkeavien solulöydösten lisäriskiä

ehkäisypillereiden käyttäjillä ei-käyttäjiin verrattuna. Päinvastoin vakiintunut ehkäisypillereiden käyttö suojasi poikkeavilta solulöydöksiltä.

Tutkimus III arvio kohdunkaulan poikkeavien solumuutosten riskiä suhteessa kuukautisten alkamiseen ja yhdyntöjen aloittamiseen, niinikään PATRICIA-trialin verrokki-armissa. Lyhyt väli kuukautisten alkamisesta yhdyntöjen aloittamiseen ei lisännyt kohdunkaulan poikkeavien solumuutosten riskiä. Sen sijaan Chlamydia trachomatis infektiosta johtuva kohdunkaulan poikkeavien solumuutosten riski oli lisääntynyt, jos väli kuukautisten alkamisesta yhdyntöjen aloittamiseen oli lyhyt.

Tutkimuksessa IV selvitimme Chlamydia trachomatis infektion ja ehkäisypillereiden käytön keston yhteisvaikutusta kohdunkaulansyövän levyepiteeliaalisten esiasteiden riskiin naisilla, jotka olivat osallistuneet suomalaiseen HPV-rokotusstrategioiden vaikuttavuustrialiin. Levyepiteliaalisten esiastemuutosten riski oli kasvanut klamydiapositiivisilla naisilla, jotka olivat käyttäneet ehkäisypillereitä viisi vuotta tai pitempään.

Tämän väitöskirjan havainnot korostavat seksuaaliterveysneuvonnan tärkeyttä, erityisesti ehkäisyneuvontaa nuorilla ja nuorilla aikuisilla naisilla. HPV tai Chlamydia trachomatis yhdessä tai erikseen aiheuttamia infektioita ei aina voida hoitaa heti. Jotkut infektioista pitkittyvät riippuen henkilön fysiologisista ominaisuuksista tai elintavoista kuten tupakoinnista, useista seksipartnereista käyttäytymisestä, ehkäisymenetelmien käytöstä ja sosiaaliluokasta. Korkean riski HPV-tyyppien aiheuttaman infection persistointi lisää vakavien kohdunkaulan solumuutosten riskiä. Vaikka HPV:hen liittyvän kohdunkaulansyövän eteneminen on pitkäkestoinen prosessi vakavien solumuutosten riski on erityisen suuri maisssa, joissa ei ole HPV-rokotusohjelmaa. Tässä tutkimuksess saatoimme todeta kohonneen levyepiteelimuutoksen (SIL) riskin HPV-rokotetuilla naisilla, joilla oli Chlamydia trachomatis infektio ja jotka olivat käyttäneet ehkäisypillereitä 5 vuotta tai pitempään. Myös korkeassa riskissä oleva rokottamattoman populaatio tulee kohtaamaan kohonneen kohdunkaulansyövän tautitaakan. Tämän vuoksi seksuaaliterveysneuvontaa pitää tukea. Prospektiivisia tutkimuksia, jotka koskevat ehkäisypillereiden käyttöä ja kohdunkaulan pahanlaatuisten muutosten riskiä tarvitaan syy-yhteyksien ymmärtämiseksi.

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ABBREVIATIONS

ADC	Adenocarcinoma
AIS	Adenocarcinoma in situ
ASC	Atypical squamous cells
ASC-US	Atypical squamous cells of undetermined significance
ASC-H	Atypical squamous cells cannot exclude HSIL
ASIR	Age-standardized incidence rate
ASMR	Age-standardized mortality rate
BMI	Body mass index
CIN	Cervical intraepithelial neoplasia
CIN1	Cervical intraepithelial neoplasia grade 1
CIN2	Cervical intraepithelial neoplasia grade 2
CIN2+	Cervical intraepithelial neoplasia grade 2 or more severe lesion
CIN3	Cervical intraepithelial neoplasia grade 3
CIN3+	Cervical intraepithelial neoplasia grade 3 or more severe lesion
CI	Confidence interval
CIS	Carcinoma in situ
CT	*Chlamydia trachomatis*
ELISA	Enzyme-linked Immunosorbent Assay
EPIC	European Prospective Investigation into Cancer and Nutrition
FMC	Finnish Maternity Cohort
FSHS	Finnish Student Health Service
FSI	First sexual intercourse
HAV	Hepatitis A-virus
HBV	Hepatitis B-virus
HDI	Human Development Index
HPV	Human papillomavirus
hrHPV	High-risk human papillomavirus
HSIL	High-grade squamous intraepithelial lesions

HSIL+	High-grade squamous intraepithelial lesions or more severe lesion
IARC	International Agency for Research on Cancer
ICC	Invasive cervical cancer
LSIL	Low-grade squamous intraepithelial lesions
MALDI-TOF	Matrix-assisted laser desorption time-of-flight
OC	Oral contraceptive
OR	Odds ratio
PCR	Polymerase chain reaction
RR	Risk ratio
SCC	Squamous cell carcinoma
SIL	Squamous intraepithelial lesions
STI	Sexually transmitted infection
THL	Finnish Institute for Health and Welfare
VLP	Virus-like particle
WHO	World Health Organization

ORIGINAL PUBLICATIONS

1. **Adhikari I,** Surcel HM, Luostarinen T, Pukkala E, Apter D, Lehtinen M. Prolonged oral contraceptive use and risk of acquisition of human papillomavirus type 16/18 infections. Submitted

2. **Adhikari I,** Eriksson T, Luostarinen T, Apter D, Lehtinen M. The risk of cervical atypia in oral contraceptive users. Eur. J. Contracept. Reproductive Health 2018; 23(1):12-17.

3. **Adhikari I**, Eriksson T, Luostarinen T, Apter D, Lehtinen M. Is the risk of cervical atypia associated with the interval between menarche and the start of sexual activity? A population-based cohort study, BMJ open 2019; 9(9): e030091.

4. **Adhikari I**, Eriksson T, Harjula K, Hokkanen M, Apter D, Niemienen P, Luostarinen T, Lehtinen M. Association of Chlamydia trachomatis infection with cervical atypia in adolescent women with short- or long-term use of oral contraceptives: a longitudinal study in HPV vaccinated women. BMJ open 2022; 12(6): e056824.

1 INTRODUCTION

Cervical intraepithelial neoplasia (CIN) is the abnormal growth of cells on the surface of the uterine cervix. CIN is an easily detectable precancerous lesion which, however, can become cancerous if left untreated (National Cancer Institute, n.d.). Worldwide, cervical cancer's highest burden is in low Human-Development Index (HDI) countries, with a global age-standardised incidence rate (ASIR) of 27.2 per 100,000 (Ferlay et al., 2020).

CIN and cervical cancer are caused by persistent infection with oncogenic human papillomavirus (HPV) types (Walboomers et al., 1999). HPV infection is a sexually transmitted infection (STI); therefore, associated risk factors are also closely related to sexual behaviour: early sexual debut, lifetime number of sexual partners and contraception/parity (IARC, 2021). HPV is considered as a necessary but insufficient cause of cervical neoplasia (Walboomers et al., 1999). Most sexually active women contract an oncogenic HPV infection(s) at one or more points in their life, but the life-time risk of developing cervical neoplasia is not more than 10% (Smith et al., 2003). Thus, there are other co-factors that independently increase the risk of cervical neoplasia and cancer, such as smoking and *Chlamydia trachomatis* (CT) infections (Kapeu et al., 2009; Lehtinen et al., 2011).

In addition to the above independent co-factors, oral contraceptive (OC) use may also play a role in cervical carcinogenesis (La Vecchia & Boccia, 2014) but the nature of the association is not fully understood. The mechanism by which hormonal contraceptives increase the risk of cervical neoplasms could be that they could promote the persistence of oncogenic HPV infections. Also, the use of OCs induces

biological changes in the cervix which fosters the progression of cervical lesions (Xu et al., 2018). This is not only the case with cervical neoplasms; but OC use is also associated with an increased risk of breast cancer (Hemminki et al., 2002) and decreased risk of uterine malignancy and ovarian malignancies (Hannaford et al., 2007). These facts suggest that OCs are potential risk modifiers for cancer.

Condoms and OCs (alone or in combination with condoms) are the most common methods of contraception among adolescents in Finland (Hassani et al., 2006). The age at sexual debut has been decreasing and the percentage who have multiple sex partners has been increasing in adolescents in the Nordic countries (Hassani, 2010). This increase in sexual activity itself reflects the need for use of contraception. The reason for the increased use of OCs among adolescents is to decrease unwanted pregnancies and abortions, but its association with risk-taking sexual behaviour is complex (Rimpelä et al., 1992).

Given the widespread use of OCs, it is important to consider the risks and benefits of OC use among women, especially teenagers, who are likely to be exposed to sexually transmitted diseases (STDs) and different cervical lesions in the long run. In this study, we examined the independent role of OC use in cervical carcinogenesis. The primary aim of the current study was to examine the role of OCs in cervical neoplastic changes. The secondary aim was to assess how other STIs, especially CT, and starting of sexual activity at an early age affects the risk of cervical neoplasia.

2 LITERATURE REVIEW

2.1 Cervical cancer

2.1.1 Natural history of cervical cancer

The cervix is the lower part of the uterus which connects the body of the uterus (the upper part where the foetus grows) to the vagina (birth canal). The cervix is made up of two different types of cells: The inner part of the cervix (endocervix) is made up of glandular cells and the outer part (ectocervix) is made of squamous cells. The place where these two cells meet is called the transformation zone (American Cancer Society, 2020). This transformation zone is more susceptible to carcinogenesis than other parts of the cervical canal.

The natural history of cervical cancer starts with a persistent infection in the cervical transformation zone with oncogenic high-risk (hr) HPV types (Schiffman & Wentzensen, 2013). These cells in the transformation zone do not change into cancer cells at once. The vast majority of women clear the HPV infection in a year or two following acquisitions. However, in a small percentage of women, the infection persists (IARC, 2003). There are different stages in cervical carcinogenesis, which begins with the HPV infection; the persistence of the HPV infection leads to the development of high-grade precancerous (precursor) lesions and invasive cancer (Schiffman & Wentzensen, 2013).

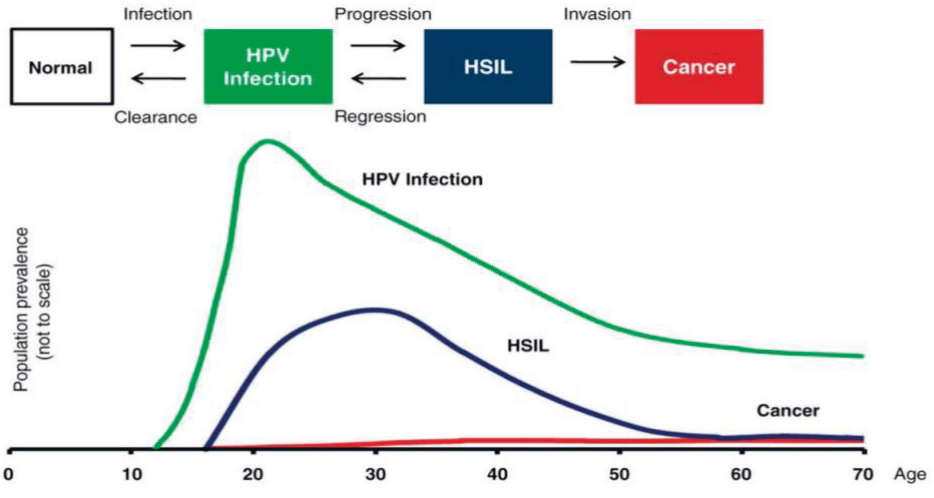

Figure 1. Natural history model and prevalence of HPV infection, HSILs and cervical cancer by age. Adapted from Schiffman and Wentzensen 2013 (89).

The precancerous lesions/changes are described using several terms, such as dysplasia, CIN, and squamous intraepithelial lesions (SILs) (American Cancer Society, 2020). These lesions are categorised from grades 1 to 3 depending upon how much of the cervical epithelium is involved: CIN1 (mild dysplasia or low-grade SIL), CIN2 (moderate dysplasia), CIN3 (severe dysplasia or high-grade SIL) and carcinoma in situ (CIS) or adenocarcinoma in situ (AIS) (Schiffman & Wentzensen, 2013). All of these stages can progress and regress naturally. CIN1 is a transient infection with HPV, CIN2 is more likely to regress than CIN3 and is considered a borderline between HPV infection and CIN3 (Schiffman & Wentzensen, 2013).

According to the Bethesda system, SILs are termed as low-grade SIL (LSIL) and high-grade SIL (HSIL), where LSIL is CIN1 and HSIL is CIN2, CIN3 and CIS. Epithelial cell abnormalities are defined as the "atypical squamous cells" (ASC) which are now qualified as "of undetermined significance (ASC-US)" or "cannot

exclude HSIL (ASC-H)" (Solomon et al., 2002). The high-grade SIL is the most serious precancerous lesion.

The progression of moderate to severe lesions takes years to decades. Women above age 35 with moderate to severe precancerous lesions are at high risk of cervical cancer (IARC, 2003). The typical time frame of the natural history of cervical cancer (Figure 1) shows the peak of HPV acquisition in adolescence and early adulthood, the peak of high-grade SIL around 25–30 years and the peak of cancer from 45 to 60 years (Schiffman & Wentzensen, 2013). The main types of cervical cancer are squamous cell carcinoma (SCC) and adenocarcinoma. Out of these two, SCC accounts for 90% of cervical cancers, and the rest are adenocarcinomas originating from the glandular cells of the cervix (Schiffman & Wentzensen, 2013).

2.1.2 Epidemiology and risk factors of cervical cancer

Cervical cancer is the fourth-most common cancer among women worldwide, with 604,127 new cases and 341,831 deaths worldwide in 2020. Both the new cases and deaths are increasing (Ferlay et al., 2020), with the highest burden of disease in East and Central Africa (Ferlay et al., 2020). The estimated age-standardised incidence rate (ASIR) is 13.3 per 100,000 women globally. Cervical cancer is the leading cause of cancer-related death among women in low- and middle-income countries, highest in the East Africa with an ASIR of 40.1 per 100,000 and age-standardised mortality rate (ASMR) of 28.6 per 100,000 women (Ferlay et al., 2020). Conversely, in the European countries, the ASIR and ASMR are 7.0 and 2.0 per 100,000 women, respectively. Within Europe, the number of new cases in the year 2020 varies from 3380 in Romania to 656 in Sweden, 397 in Norway and 185 in Finland (Ferlay et al., 2020). In Finland, the incidence of cervical cancer is lower than in other Nordic countries (NORDCAN, 2019). The aim of the World Health Organization (WHO) is to reduce the ASIR of cervical cancer to less than 4 per 100,000 women worldwide by vaccinating 90% of all girls by age 15 years, screening 70% of women twice in the age range of 35–45 years and treating at least 90% of all precancerous lesions detected during screening (Arbyn et al., 2020).

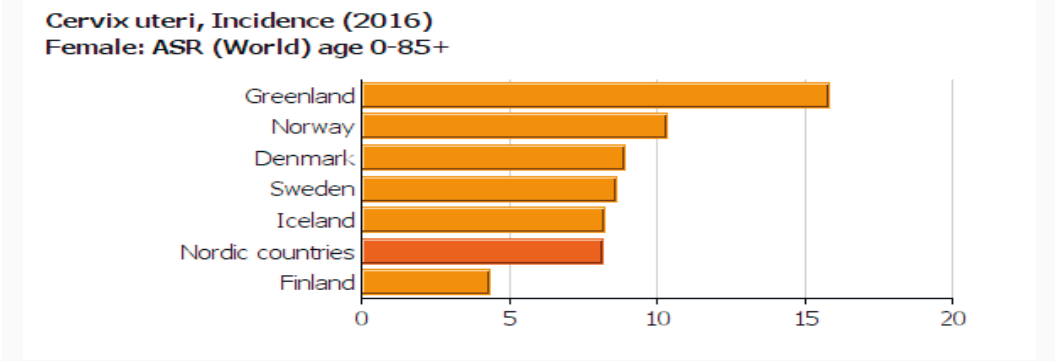

Figure 2. Incidence of cervix uteri in Nordic countries. Adapted from NORDCAN 2016 (74).

The primary cause in the development of cervical cancer is human papillomavirus (HPV), most notably HPV type 16 (Durst et al., 1983; Hausen et al., 1974; Walboomers et al., 1999). In the past, before HPV discovery, there were different assumptions about the causes of cervical cancer. Some observations helped to shape the causal framework of cervical cancer (Gagnon, 1950). In 1974, Valerie Beral published a paper on the aetiology of cervical cancer where she demonstrated that cervical cancer is sexually transmitted. She found that the incidence and mortality pattern of cervical cancer are similar to those of Gonorrhoea. Thus, women who had an early age of sexual debut have a risk of sexually transmitted diseases (Beral, 2015). Later on, and until now, there have been numerous studies which have proved that there are behavioural and sexual factors which increase the risk of HPV persistence and eventually cervical cancer, such as early sexual debut, multiple number of sexual partners (Dillner et al., 1996; Dillner et al., 1999), multiparity, smoking (Kapeu et al., 2009), Chlamydia trachomatis infection (Lehtinen et al., 2011) and use of oral contraceptives (Smith et al., 2003) (Franceschi & Vaccarella, 2015).

2.1.2.1 Human papillomavirus infection

In 1974, Dr Harald zur Hausen and his team discovered HPV DNA for the first time in plantar warts. But that HPV DNA was not found in cervical cancer biopsies and genital warts (Hausen et al., 1974). However, this was the hint that there could be different types of HPV DNAs. In 1979, Dr zur Hausen and his team successfully cloned HPV 6 DNA for the first time. Then they also isolated and cloned HPV16 and HPV18 within some years (Boshart et al., 1984; Boshart et al., 1985; Durst et al., 1983). Thus, Dr zur Hausen proved his initial hypothesis that cervical cancer is caused by an infectious agent i.e., HPV (Hausen et al., 1987). Later, there have been many epidemiological studies which have already proved that genital HPV is the central factor in the aetiology of cervical cancer worldwide (Syrjanen et al., 1990; Dillner et al., 1997; Lehtinen et al., 1996).

There are over 200 types of HPV, but the carcinogenic types include HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59 and HPV68 (Schiffman et al., 1993). The most important HPV type is HPV16, which causes about 50% of cervical cancers, and the next most important is HPV18, mostly responsible for adenocarcinoma (Durst et al., 1983; Boshart et al., 1984). Genital HPV is transmitted through sexual contact. The highest rates of genital HPV infection have been reported in sexually active women aged <25 years. The highest prevalence of HPV infection in women is in their 20s (Steben & Duarte-Franco, 2007). The global prevalence of HPV infection based on testing over 1 million women using polymerase chain reaction (PCR) was 11–12%, with the highest prevalence in sub-Saharan Africa (24%), Eastern Europe (21%) and Latin America (16%) (Forman et al., 2012).

2.1.2.2 Human papillomavirus infection and cervical cancer

Over the years, there have been many epidemiological studies which have shown that HPV infection causes virtually all cases of cervical cancer and preinvasive precursors. Some of these are discussed here.

In 1993, a large case-control study was published which evaluated how sexual behaviours and other risk factors of CIN influence the risk via HPV infection. Schiffman et al. studied CIN as a surrogate endpoint for cervical cancer using 500 women with CIN and 500 control subjects. Most grades of CIN could be attributed to HPV infection. Moreover, the case subjects had more cigarette smoking, more sex partners, an earlier age at first sexual intercourse and lower socioeconomic status (Schiffman et al., 1993). Likewise, Bosch et al. investigated whether the association between cervical cancer and HPV is similar worldwide. They analysed more than 1000 frozen samples from 22 countries and found that HPV DNA was detected in 93% of the tumours, and no significant variation in HPV positivity among the countries was detected. About 50% of the specimens were HPV16 positive (Bosch et al., 1995)

In the largest nested case-control study among the Nordic population, the role of HPV infection in cervical carcinogenesis was studied. Dillner et al. followed up a joint cohort of 700,000 Nordic women for 5 years. Serum samples of the women who developed invasive cervical cancer during the follow-up time and the control women were analysed. The study found an increased risk of SCC associated with HPV16 seropositivity, and the increased risk of adenocarcinoma was associated with HPV18 seropositivity (Dillner et al., 1997). Furthermore, among a Nordic population, one study (Luostarinen et al., 1999) investigated the joint effect of oncogenic and non-oncogenic HPV types and their risks of developing cervical cancer. A cohort of 530,000 women who had donated their serum samples to Nordic serum banks (in Finland, Norway and Sweden) were linked with their respective

cancer registries. Luostarinen et al. found an antagonistic interaction between HPV16 and HPV6/11 in cervical carcinogenesis which means there is no excess risk of cervical carcinoma in women positive for both HPV16 and HPV6/11 (Luostarinen et al., 1999).

A population-based prospective study was performed in Sweden with the aim of observing the risk of invasive cervical cancer among healthy women with normal cervical cytological findings positive for HPV DNA. The women participating in the population-based cervical cancer screening programme were followed up for 5.6 years. Wallin et al. found an increased risk of invasive cancer in the HPV-positive women. The HPV DNA test can predict the risk of cervical cancer in women with normal pap smears (Wallin et al., 1999).

In 1996, Lehtinen et al. conducted a nested case-control study to study the role of HPV type 16 in the aetiology of cervical carcinoma. The study included a cohort of 18814 Finnish women who participated in a mobile health examination survey in different parts of Finland. Seventy-two cases of cervical carcinoma (27 ICC and 45 CIS) and 143 matched controls were enrolled in the study. Lehtinen et al. found an increased risk of cervical carcinoma associated with HPV16. After adjusting for smoking and antibodies to various other agents of sexually transmitted diseases, the association of HPV16 with cervical carcinoma remained significantly strong (OR: 12.5, 95% CI: 2.7–57, $P<0.001$) (Lehtinen et al., 1996).

Similarly, the association between HPV16 and cervical carcinoma in situ was studied by Ylitalo et al. in a nested case-control study where they reported a high viral load of HPV16 in 478 women with cervical carcinoma in situ. The women with a high viral load of HPV16 had 30 times higher relative risk compared to HPV-negative women. About 25% of the women infected with a high viral load before age 25 developed CIS within 15 years (Ylitalo et al., 2000).

A systematic review and meta-analysis were done by Koshiol et al. to study the association between persistent HPV DNA and high-grade CIN2–3, HSIL and invasive cervical cancer. HPV persistence was defined as HPV positivity at two or more times. This study reported HPV persistence as a clinical marker and endpoint. HPV persistence was consistently and strongly associated with CIN2–3/HSIL+. Koshiol et al. also emphasised the importance of the precise definition and standardisation of HPV testing, sampling procedures and test intervals for reliable clinical inferences (Koshiol et al., 2008).

2.1.2.3 Chlamydia trachomatis and cervical cancer

Chlamydia trachomatis (CT) is one of the most common sexually transmitted bacterial infections (Paavonen, 2001). CT bacteria infect genital tissues, induce chronic inflammation and damage epithelial tissues (Smith et al., 2004). CT is common in sexually active young women with an early age at first intercourse, multiple sex partners and use of non-barrier contraceptive methods (Handsfield et al., 1986). In 2020, the WHO estimated 129 million new infections of CT (WHO, 2020). In Finland, 16,790 Chlamydia infections were found in 2021, which is higher than the previous year. Out of these, 58% of the infections were concentrated in women and 79% in young people of age 15–29 years (THL, 2022). CT infection is also known as a marker of sexual activity and the major cause of mucopurulent cervicitis, pelvic inflammatory disease, tubal factor infertility and ectopic pregnancy (Paavonen, 2001). Among sexually transmitted infections, besides HPV, CT has been identified as an independent cofactor for CIN3 (Lehtinen et al., 2011).

The International Agency for Research on Cancer (IARC) study examined the role of CT infection as an HPV cofactor associated with the risk of invasive cervical cancer (ICC). Smith et al. found an increased risk of squamous cervical cancer associated with CT infection among HPV-positive women (Smith et al., 2002). In a prospective sero-epidemiologic study, Koskela et al. observed that 30% of 149 cervical SCC cases, compared with 13% of 442 controls, were seropositive for CT at baseline. Serum antibodies to CT were associated with the highest risk of SCC (OR: 2.2, 95 % CI: 1.3–3.5), and the association remained after adjusting for smoking and serum antibodies to high-risk HPV types (Koskela et al., 2000). Luostarinen et al. conducted a longitudinal study among a cohort of 94,349 Finnish women to investigate the order of HPV/CT infection on the risk of high-grade cervical pre-cancer. The study found a very high relative risk of CIN3/HSIL for concomitant CT and high-risk HPV 18 and 45 (rate ratios: 28, 95 % CI: 4.3–190) compared to HPV 18 and 45 and CT seropositive at baseline (Luostarinen et al., 2013).

To determine whether the risk of SCC is serotype-specific, a longitudinal study was conducted using the Nordic serum samples from a serum bank. CT serotype G was strongly associated with cervical SCC (Anttila et al., 2001); also, increasing numbers of exposures to different CT serotypes increased the risk of cervical SCC. Wallin et al. showed that past CT infection was related to an increased risk of ICC (OR: 17.1, 95% CI: 2.6–∞) in a population-based prospective study using PCR analysis (Wallin et al., 2002).

A cross-sectional IARC study of 1,238 cases of ICC and 1,100 control women from 7 countries found the risk of ICC increased in CT-seropositive women (OR: 1.8, 95% CI: 1.2–2.7); CT antibodies were associated neither with adenocarcinoma (ADC) nor with adenosquamous carcinoma (OR: 1.0, 95% CI: 0.53–1.9) (Smith et al., 2004). In a population-based cross-sectional study of 302 women with invasive SCC, 185 women with adenocarcinoma of the cervix and 318 HPV-seropositive control women, Madeleine et al. observed an increased risk of SCC associated with CT antibodies (OR: 1.6, 95% CI: 1.1–2.2), but not of adenocarcinoma (OR: 1.0, 95% CI: 0.6–1.5) (Madeleine et al., 2007).

The association between cervical cancer and CT may be due to an influence of CT on the persistence of high-risk HPV. Samoff et al. investigated whether different genital tract infections such as CT*, Neisseria gonorrhoeae, Trichomonas vaginalis* and bacterial vaginosis are associated with HPV persistence using PCR assays. Concurrent infection with CT was independently associated with the persistence of high-risk HPV types (adjusted OR: 2.1, 95% CI: 1.0, 4.1) (Samoff et al., 2005). On the other hand, Deluca et al. found an increased risk of CT infection in HPV DNA-positive Argentine women (OR: 2.2, 95% CI: 1.2–4.3) compared to HPV DNA-negative women by PCR analysis (Deluca et al., 2011). However, Lehtinen et al. observed that CT was associated with CIN2 among women both with (hazard ratio: 1.82, 95% CI: 1.06–3.14) and without HPV 16/18 infection (hazard ratio: 1.74, 95%

CI: 1.05–2.90). Serology was not performed in this study (Lehtinen et al., 2011). Thus, CT status may be independently involved in the early stages of cervical carcinogenesis.

Seraceni et al. investigated HPV prevalence and its genotype distribution in women at risk of CT infection by using real-time PCR analysis. The prevalence of multiple HPV infections was high among young women with chronic CT infections. Chronic CT infection was defined as the presence of a large quantity of heat shock protein 60 (Hsp 60) in the bloodstream, which could be detected by PCR (Seraceni et al., 2014). Next, in the women with persistent HPV infections, the risk of CIN3+ is higher if there are repeated CT infections compared to a single infection. Also, CT infection at a young age is associated with an increased risk of cervical neoplasia, in addition to HPV persistence (Jensen et al., 2014). Thus, the biological effect of CT may be to damage the mucosal barrier, strengthen the HPV infection, and interfere with the immune response and viral clearance, thus helping HPV persistence (Silva et al., 2013).

Most of the above-mentioned studies have performed *Chlamydia* serology tests using microimmunofluorescence (MIF) (some have also used enzyme-linked immunosorbent assays, EIA) which are CT serotype-specific. The rest of them only used *Chlamydia* DNA detection tests (PCR). While these studies have supported an association between CT and HPV infections and cervical neoplasia, there are also some studies which have not found any significant association between CT antibodies and cervical premalignancy or cervical cancer (Ferrera et al., 1997; Safaeian et al., 2010).

2.1.2.4 Oral contraceptives and cervical cancer

Oral contraceptives (OCs) are the most common method of contraception among women (Baird & Glasier, 1993; Cooper & Mahdy, 2021). There are three main types of OC pills: combined oestrogen-progesterone, progesterone only and the continuous or extended-use pill (Cooper & Mahdy, 2021). Among these, the combined OC pills are used commonly to prevent pregnancy. These pills work by inhibiting ovulation and thickening the cervical mucus and making it unlikely for the sperm to penetrate the cervix (Cooper & Mahdy, 2021). The development of OCs in the 1950s was a landmark in controlling human fertility, and it has allowed women to fully participate in society (Baird & Glasier, 1993). This is also why the OC pills have been a popular choice of contraception among women in Western countries (Baird & Glasier, 1993; Cooper & Mahdy, 2021).

The fact that OC pills can also be used for emergency contraception after unprotected sex has during the last 10 years made them a popular choice of contraception (WHO, 2021). However, after the widespread use of OC pills, there are concerns about the safety of using such treatment uncontrolled.

The use of OCs has been suspected to be associated with cervical cancer in many epidemiological studies (Delgado-Rodriguez et al., 1992). In one IARC study, the risk of cervical cancer associated with OC use was studied among women who tested positive for HPV DNA. Moreno et al. pooled data from eight case-control studies of invasive cervical cancer patients and two studies of carcinoma in situ patients. The risk of cervical cancer increased with the increase in the duration of OC use. For those who had used OCs for 5–9 years, the odds ratio was 2.8 (95% CI: 1.5–5.4), and for those who had used them for 10 years or more, the risk was 4.0 (2.1–8.0). The study concluded that the long-term use of OCs could be a cofactor in increasing the risk of cervical cancer in HPV-positive women (Moreno et al., 2002). Similarly, Smith et al. found the risk of cervical cancer increased with the duration of OC use.

They reviewed 28 studies which included 12,531 cases of cervical cancer. The relative risk of cervical cancer increased with duration: 1.1 (95% CI: 1.1–1.2) for less than 5 years of OC use, 1.6 (1.4–1.7) for 5-9 years of OC use and 2.2 (1.9–2.4) for 10 years or more (Smith et al., 2003).

A similar pattern was observed in another pooled analysis of 24 different studies to investigate the association between cervical carcinoma and OC use. This study also found that the risk of cervical cancer increases with the increase in the duration of OC use and declines after use ceases (Appleby et al., 2007). Many epidemiological studies have suggested that the risk of precancer lesions increases with the duration of OC use and decreases with the cessation of use (La Vecchia & Boccia, 2014; Oh et al., 2016; Roura et al., 2016). On the contrary, in one South African study, Shapiro et al. did not find any association between the oestrogen-progesterone combined pill or progesterone-only pills and invasive cervical cancer (Shapiro et al., 2003). The relative risk of invasive cervical cancer from OC use was 0.8 (0.7–1.1), which remained almost the same after adjusting for possible confounders (Shapiro et al., 2003). In the similar contexts, other epidemiological studies have also found no increased risk of cervical cancer/lesions due to OC pills (Longatto-Filho et al., 2011; Peng et al., 2017).

The risk of cervical neoplasia (HSIL) is higher in current OC users compared to non-users (Xu et al., 2018). In a recent study among current users of hormonal contraceptives, the risk of developing high-grade CIN increased by 50% compared to women who had never used them or used them in the past. Also, the current users were at increased risk of cervical neoplasia with increased duration of hormonal contraceptives (Xu et al., 2018). On the contrary, it has been also found that there may be no need to stop the use of OCs before or after the diagnosis of HPV lesions/CIN lesions and their treatment. In fact, the use of OCs has been suggested to increase compliance with treatment and follow-up (Frega et al., 2007). Frega et al.

evaluated the effect of oral contraceptive use on the recurrence rate of HPV lesions and CIN lesions following the ablative or excisional procedure. OC use did not increase the recurrence rate of HPV lesions and/or CIN after ablative or surgical treatment (Frega et al., 2007).

The mechanisms of why OC use increases the risk of cervical neoplasia is not clear. Progesterone reacts with the hormone receptors present in the cervical tissue to influence the natural history of HPV infection (Roura et al., 2016). OC use does not provide protection against sexually transmitted infections. OC users have inconsistent condom use; thus, they easily acquire the HPV virus (Cromwell & Daley, 2000). Mostly, epidemiological evidence has suggested that OC use promotes the persistence of oncogenic HPV infection, thereby increasing the progression of cervical cancer (Nielsen et al., 2010; Salazar et al., 2005).

In their prospective study among Danish women, Nielsen et al. had investigated the risk factors for the persistence of HPV infection. Ever having used OCs was found to be associated with an increased risk of persistent high-risk HPV infection (Nielsen et al., 2010). In another large study of OC use and HPV positivity, Vaccarella et al. concluded that the use of OCs might help in the malignant transformation of HPV infections to cervical lesions rather than helping in the persistence of HPV infection (Vaccarella et al., 2006). In another study, the use of the combined OC pill for longer durations (>6 years) was associated with prevalent HPV infection, independent of sexual and cervical abnormalities, as compared to never users (Marks et al., 2011).

One nested case-control study examined the risk factors for non-oncogenic, oncogenic and HPV16 cervical infections. During the analysis, the women who developed infections with exclusively non-oncogenic types and any oncogenic type or HPV16 were compared with the women who were HPV-negative throughout the follow-up period of 1 year. OC use was strongly and exclusively associated with oncogenic HPV and HPV16 infections (Rousseau et al., 2000).

2.1.2.5 Smoking and cervical cancer

In 1977, Winkelstein was the first to hypothesise that smoking may be strongly associated with cervical cancer (Winkelstein, 1977). Since then, many epidemiological studies have identified smoking as a cofactor of cervical cancer (Deacon et al., 2000; Kapeu et al., 2009). In a large Nordic joint study, a nested case control study examined the independent role of smoking in cervical carcinogenesis. In this study, five large Nordic population-based serum banks collaborated for the retrieval of serum samples. The serum samples were analysed for cotinine, which is a marker for current tobacco exposure, and antibodies to HPV types 16 and 18, herpes simplex virus type 2 and CT. The study found that smoking is associated with the risk of SCC among both HPV16- and/or HPV18-positive and negative heavy smokers, with overlapping risk estimates and confidence intervals with an odds ratio 2.7 (95% CI: 1.7, 4.3) and an odds ratio of 3.4 (95% CI: 2.5, 4.4) respectively. They found a dose-response relation between cotinine level and the risk of cervical cancer in both HPV16/18-seropositive women and HPV16/18-seronegative women. The risk of SCC was high (OR: 3.2, 95% CI: 2.6, 4.0) even after adjustment for HPV 16/18 in heavy smokers (Kapeu et al., 2009). In one EPIC (European Prospective Investigation into Cancer and Nutrition) cohort study, Roura et al. studied the association between smoking and CIN3/CIS and ICC in a nested case-control setting. The participants were followed up for a mean of 9 years. The smoking associated risk was two-fold for both CIN3/CIS and ICC. Again, the risk was consistent even after adjusting for HPV and CT (Roura et al., 2014).

In yet another longitudinal study, Collins et al. found in the analysis of 1485 women aged 15–19 years that current smoking intensity is associated with CIN 2/3. After adjustment of HPV status, the hazard ratio for 10 or more cigarettes per day was 2.2 (95% CI: 1.2–4.1). In women who were HPV-negative and cytologically normal at recruitment, current smoking was not significantly associated with cervical HPV infection after controlling for the lifetime number of sexual partners (Collins et al.,

2009). Costa Rican HPV-positive women with HSIL+ were compared to HPV positive women without HSIL+. The relative risk of HSIL+ was 2.7 (95% CI: 1–6.7) for women who smoked 6+ cigarettes/day compared to non-smokers (Hildesheim et al., 2001). Moreover, there are other studies which found an excess risk of CIN that persisted even after controlling for HPV infection (Becker et al., 1994; Deacon et al., 2000; Kjellberg et al., 2000).

Along with active smoking, passive smoking has also been proven to increase the risk of cervical neoplasia (Trimble et al., 2005). Trimble et al. used a cohort from census data from 1963 and 1975 in Washington. The adjusted relative risk of passive smoking was 2.1 (95% CI: 1.3, 3.3) in the 1963 cohort and 1.4 (95% CI: 0.8, 2.4) in the 1975 cohort. The adjusted relative risk for current smoking was 2.6 (95% CI: 1.7, 4.1) and 1.7 (95% CI: 1.1, 2.6) in the 1963 and 1975 cohorts, respectively (Trimble et al., 2005). On the contrary, studies have also found no excess risk of SCC and adenocarcinoma associated with current smoking (Brinton et al., 1987; Lacey et al., 2001). In a multi-centre case-control study, Lacey et al. studied about cervical adenocarcinoma. After controlling for HPV, education, number of sexual partners and screening history, smoking was not associated with adenocarcinoma (OR: 0.8, 95% CI: 0.5–1.2) or SCC (OR: 1.4, 95% CI: 0.8–2.3) (Lacey et al., 2001). Thus, we can say that even though HPV is the main causal factor of both SCC and adenocarcinoma, other aetiologic co-factors contribute, but to variable degrees (Lacey et al., 2001).

As for the biological mechanism by which smoking may contribute to the risk of cervical cancer, the cervical mucus of smokers contains cigarette constituents and their metabolites (Fonseca-Moutinho, 2011). This can promote the malignant transformation of endocervical cells, e.g., via genotoxic damage of DNA detectable in cervical exfoliated cells (Plummer et al., 2003). Further, smoking also affects the local immune mechanism in the cervix (Plummer et al., 2003). Reduction or cessation

of smoking has been found to be beneficial in early cervical abnormalities (Szarewski et al., 1996). The effect of cessation was a reduction in lesion size of at least 20% among the women who quit or reduced smoking by more than 75% for 6 months compared to the non-quitters (OR: 12.0, 95% Cl: 3.9–32.7) (Szarewski et al., 1996).

3 THESIS OBJECTIVES

The overall aim of this thesis was to find out 1) whether the use of oral contraceptives is associated with the development of cervical neoplasia and 2) how the use/duration of oral contraceptive use and other sexual behaviours are associated with the risk of HPV and *C. trachomatis* infection, eventually cervical neoplasia.

The specific aims of the individual studies were:

Paper I: To assess the risk of persistent HPV infection based on the duration of oral contraceptive use

Paper II: To examine the risk of cervical atypia in oral contraceptive users.

Paper III: To examine how the changing interval between the start of sexual activity and menarche changes the risk of cervical atypia.

Paper IV: To assess the risk of cervical atypia from the combined effect of *Chlamydia trachomatis* and duration of oral contraceptive use.

4 MATERIAL AND METHODS

The first paper gets its data from the Finnish Student Health Service (FSHS) and the Finnish Maternity Cohort (FMC) serum bank. The data in the second and third papers included in this thesis stem from a randomised phase III trial of HPV16/18 vaccine efficacy (Lehtinen et al., 2012; Paavonen et al., 2007). The fourth paper gets its data from a community randomised HPV vaccination effectiveness trial in Finland (Lehtinen et al., 2014; Lehtinen et al., 2018).

4.1 Finnish Student Health Service (FSHS)

The FSHS, earlier known as the Helsinki Student Health Service (HSHS), was established in 1954. The purpose of the FSHS is to provide health services to those studying for a bachelor's or master's degree at a university or other institution of higher education. It is compulsory for all students to register with the FSHS, and they are obliged to pay the health service fee (FSHS, n.d.).

The student health care services include monitoring and promotion of students' health and welfare and fitness to study, health check-ups according to individual needs, medical care services, mental health and substance abuse services, promotion of sexual health, oral health care, early identification of any special needs and tests required by students and referral, if necessary. The FSHS activities are funded by healthcare fees and service fees paid by students (23% of funding) and reimbursements paid by the Social Insurance Institution of Finland (Kela; 73% of funding) (FSHS, n.d.).

4.2 Finnish Maternity Cohort (FMC) serum bank

The Finnish Institute for Health and Welfare (THL) established the Finnish Maternity Cohort (FMC) serum bank in Oulu in 1983. It stores the countrywide serum samples of all pregnant women in Finland. The samples are drawn at maternity clinics from women during early pregnancy (first trimester) in order to screen for congenital infections. Altogether, 2 million samples from 1 million women are stored at -25°C in the serum bank (Koskela et al., 2000; Lehtinen et al., 2017).

4.3 Randomised controlled PATRICIA trial (Phase III) of HPV16/18 vaccine efficacy

Between May 2004 and June 2005, healthy women aged 15–25 years were enrolled in this trial in 135 centres in 14 countries (Australia, Belgium, Brazil, Canada, Finland, Germany, Italy, Mexico, Philippines, Spain, Taiwan, Thailand, UK, and USA) to assess the efficacy of the HPV 16/18 vaccine (Paavonen et al., 2007). Eighteen thousand, six hundred, forty-four women who reported no more than six lifetime sexual partners before the study enrolment (in some countries, these criteria were not considered for minors), agreed to use adequate contraception over the vaccination period and had an intact cervix were eligible for inclusion. Women were enrolled irrespective of their HPV DNA status, HPV serostatus or cytology at baseline. Those women with a history of colposcopy, who were pregnant or breastfeeding, and who had chronic autoimmune disease or immunodeficiency were excluded from the study (Paavonen et al., 2007; Lehtinen et al., 2012). Written informed consent was obtained from all the participants. For minors, informed assent with written consent was obtained from a parent or legal representative. The protocol and other materials were approved by independent ethics committees or institutional review boards in each location (Lehtinen et al., 2012). This trial was

registered with the US National Institutes of Health clinical trial registry, number NCT00122681.

The women were randomly assigned in a 1:1 ratio to receive either HPV-16/18 AS04-adjuvanted vaccine (Cervarix, GlaxoSmithKline Biologicals, Rixensart, Belgium) or a control hepatitis A vaccine (GlaxoSmithKline Biologicals) at 0, 1 and 6 months. The trial remained double-blinded for 48 months after the first vaccination. After 48 months, both groups were offered cross-vaccination (Paavonen et al., 2007). The vaccines were identical in appearance and provided in prefilled syringes. Cervical liquid-based cytology samples were obtained from all women every 6 months for HPV DNA typing. Broad-spectrum PCR SPF_{10}-$LiPA_{25}$ (version 1 based on licensed Innogenetics SPF10 technology; Labo Biomedical Products, Rijswijk, Netherlands) was used to test cervical and biopsy samples for the presence of DNA from 14 oncogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) (Lehtinen et al., 2012).

Gynaecological and cytopathological examinations were done every 12 months. A prespecified clinical management algorithm for abnormal cytology results and colposcopy referral was used (Lehtinen et al., 2012). Colposcopy was recommended after two consecutive or intermittent reports of oncogenic HPV DNA-positive (with Hybrid Capture 2, Gaithersburg, MD, USA) atypical squamous cells of undetermined significance, low-grade squamous cell intraepithelial lesion (independent of HPV DNA results) or one report of atypical glandular cells, high-grade squamous intraepithelial lesion, or atypical squamous cells in which high-grade squamous intraepithelial lesions could not be excluded. The protocol also permitted referral for colposcopy after a single diagnosis of oncogenic HPV DNA-positive atypical squamous cells of undetermined significance or a cytological diagnosis of low-grade squamous intraepithelial lesions. Biopsy was recommended for any suspected cervical lesions at colposcopy. CIN2+ (defined histologically as CIN2, CIN3 or adenocarcinoma in situ) was treated by conisation (Paavonen et al., 2007).

A central laboratory (Quest Diagnostics Clinical Trials, Teterboro, NJ, USA) processed and interpreted results from liquid-based cytology and histology samples. All CIN cases were confirmed by an expert histopathology review panel that was blinded to vaccine status, HPV DNA status before biopsy and cytology reports. HPV DNA testing was done at DDL Diagnostic Laboratory (Voorburg, Netherlands) (Paavonen et al., 2007).

4.4 Community randomised (Phase IV) HPV vaccination effectiveness trial

In 2007–2009, a community randomised trial was launched among the adolescents from 33 different communities in Finland to assess the effectiveness of gender-neutral vs. girls-only vaccination (Lehtinen et al., 2014). All 80,272 Finnish-or Swedish-speaking boys and girls in the 1992–1995 birth cohorts were invited during two school years (2007–2008 and 2008–2009) by letters sent to their parents or legal guardians. The adolescent girls and boys were identified by the Finnish Population Information System maintained by the Population Register Centre. The letters included trial information, a consent form, and a prepaid return envelope for the consent. The 33 communities were randomised into three study arms, 11 in each arm (Lehtinen et al., 2014). HPV16/18 seroprevalence was determined in 50 randomly selected <23-year-old women per community. Before randomisation into the three study arms, the 33 communities were stratified into three groups according to HPV-16/18 seroprevalence: low (<20.5%, 12 communities), intermediate (20.5–24%, nine communities) and high (>24%, 12 communities). Ethical clearance was obtained from the Ethical Review Board of the Pirkanmaa Hospital District (Eudra-CT number 2007-001731-55) (Lehtinen et al., 2014, 2018).

The first appointment/study visit took place at the school health care facilities of 250 municipal junior high schools. In Arm A, 90% of participants received AS04-HPV16/18 vaccine (Cervarix®), and 10% received hepatitis B-virus, HBV vaccine (Engerix™ B). In Arm B, 90% of females received HPV-16/18 vaccine, and 10% of females and all males received HBV vaccine. In Arm C communities, all participants received HBV vaccine. The remaining two vaccine doses (at months 1 and 6) were given at schools by the same study nurses. All the subjects in Arm A and girls in Arm B were blinded. The blinding was maintained until 18.5 years of age (Lehtinen et al., 2018).

In 2010–2014, all the 1992–1995-born female residents in the trial communities were invited at the age of 18.5 years for a follow-up visit. They were offered cross-vaccination with either HPV16/18 vaccine or HBV vaccine, whichever they had not received earlier in the trial. A cervical cytological sample taken by a study nurse and a self-collected cervico-vaginal sample for HPV and/or *C. trachomatis* testing were obtained. All the female participants during the follow-up agreed to participate in a *C. trachomatis* screening trial and filled in a questionnaire on demographics, life-habits, and sexual behaviour (Lehtinen et al., 2018). Four years later (2014–2018), all the HPV16/18-vaccinated female participants were invited for a second follow-up visit at age 22 years of age (Louvanto et al., 2020). Again, a cervical cytological sample and a self-collected cervico-vaginal sample for HPV and/or *C. trachomatis* testing were obtained, and the participants again filled in the questionnaires (Lehtinen et al., 2014).

All self-collected samples were analysed by PCR, with HPV typing, using matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry for detection of HPV6/11/16/18/31/33/35/39/45/51/52/56/58/59/66/68. The DNA sequence specific to the presence of *C. trachomatis* in the sample was detected by commercial PCR (Abbott-TM) (Lehtinen et al., 2018).

4.5 Study Design and Population

4.5.1 Paper I

This study is nested (nested case control) in a Finnish Student Health Service (FSHS) cohort of 37,153 female university students. The study cohort comprises two parts: 1) female students from the University of Helsinki (n=28,109), and 2) other female university students (n=9,044) in the Helsinki area, born in 1946–1960, who used the FSHS from 1965 and 1980 onwards, respectively. Data on OC and alcohol use, menarche, parity, smoking and body mass index (BMI) were collected and abstracted from the FSHS patient records for 706 women, of whom 316 served as controls for an earlier breast cancer and OC study (Hemminki et al., 2002), with a minimum of three visits to FSHS. To study HPV infections, archived serum samples for the FSHS cohort were retrieved from the FMC serum bank. Altogether, 565 serum samples were identified for 297 women of the original 706 women devoid of an early breast cancer diagnosis. The first pregnancy serum samples were retrieved for antibody analysis. The ethical review board of the Northern Finland Hospital District approved the present study.

4.5.2 Paper II and Paper III

For both papers, all the women who had received the control (HAV) vaccine in the PATRICIA trial were included. Both of these studies were longitudinal in design. These women had answered the questionnaires both at enrolment at age 16–17 and at the end of the follow-up when they were 22 years old (Figure 3). The questionnaires had information on smoking, menarche, sexual habits, history of OC use and the use of other contraceptives. The data on cytology were also available for these women from the cervical cytological samples taken every 6 months during the trial period. In Study II, only the women who had filled questionnaires and had

negative cytology at baseline were included, whereas in Study III, the women who had negative cytology at baseline and before menarche in addition to questionnaires were included.

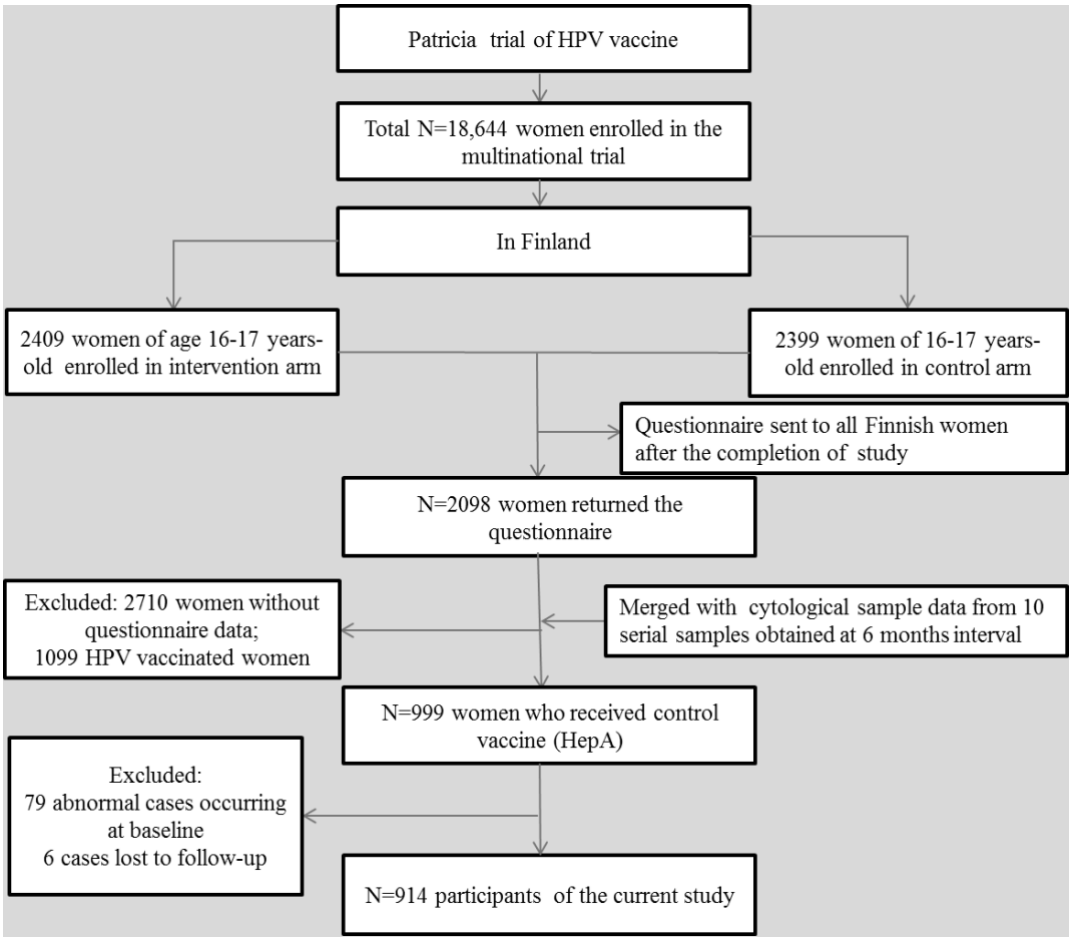

Fig 3: Flow diagram of study subject's selection. Adapted from paper II

4.5.3 Paper IV

For this longitudinal study, we used a data from a community randomised HPV vaccination effectiveness trial. We included all the women who attended the *C. trachomatis* screening and filled in the sexual and behavioural questionnaires at 18.5 and 22 years of age (Figure 4). HPV DNA data and cytology data were also available for these participants at both 18.5 and 22 years of age. We merged all the four datasets using personal IDs and included only those that merged. All those which did not match/merge with any of the datasets were excluded from the study at the end of merging (Figure 4). The final number of participants in this study at 18.5 years of age was 11,701 and at 22 years of age was 6618 (Figure 4).

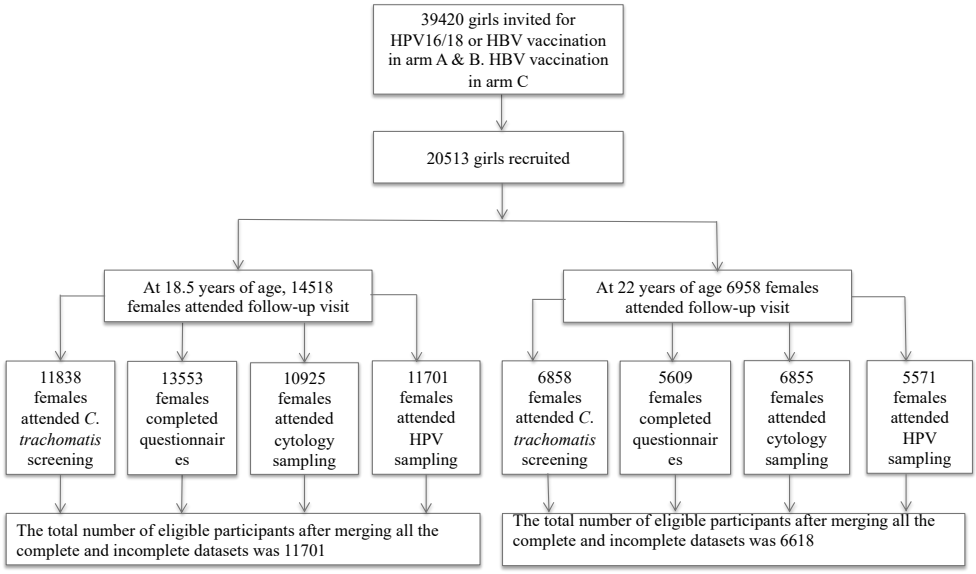

Figure 4. Flow diagram of female community randomized trial participants. Adapted from paper IV

4.6 Statistical analyses

4.6.1 Paper I

Serologically defined HPV16 and HPV18 infections were the main outcome variables. They were detected through antibody analyses done with a standard virus-like particle (VLP) enzyme-linked immunosorbent assay (ELISA) test. HPV16 and HPV18 VLPs were used for the detection of HPV16 and HPV18 antibodies.

C. trachomatis antibodies were measured by a commercial major-outer membrane protein-derived peptide-ELISA, as described by Luostarinen et al. (2013). *C. trachomatis* antibody results were classified into three categories: negative, equivocal, and positive. Cut-off levels of 1.0 and 1.4 were applied for equivocal and positive, respectively.

OC use was categorised according to the duration of use into three categories: non-users, OC use less than 5 years (<5 years) and OC use for 5 or more years (≥5 years). Age at serum sampling was divided into four categories: less than 30, 30–34, 35–39 and 40–44. Smoking was classified into three different categories: non-smokers, current smokers, and past smokers. Parity was categorised into two groups: one group having fewer than 4 children and another group with 4–7 children. Body mass index (BMI) was categorised into four different categories: underweight (<18.5), normal weight (18.5–24.9), pre-obesity (25.0–29.9) and obese (≥30) (WHO, 2010).

The relative risk of HPV infection associated with duration of OC use was calculated as OR with 95% CI using Stata version 13.0. The crude OR was adjusted for age, smoking and *C. trachomatis* antibodies using logistic regression. Confounding by parity was not controlled for, as parity was affected by the exposure and duration of OC use.

4.6.2 Paper II

The use of OCs in women diagnosed with SIL was the main variable of interest. The first findings of atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL) were registered as index incident cases for the statistical analysis. Colposcopy-directed biopsy samples were obtained during the trial. The first histopathological findings of CIN grades 1, 2 and 3 were listed as the index cases for statistical analysis. Information on *C. trachomatis* testing at the study visits was available for adjusting the statistical analysis.

The incidence rates of cytological and histopathological abnormalities were calculated with 95% confidence intervals (95% CI) and expressed per 100 person-years using the concept of survival analysis. The relative risk of cervical atypia was calculated as odds ratios (OR) with 95% CI using logistic regression in Stata version 14.0 (Stata Corp LP, Statistical Software: Release 14, College Station, TX, USA).

4.6.3 Paper III

In this study, the main outcome variable was cervical atypia. The ages at the start of OC use, menarche, and age at first sexual intercourse (FSI) were the independent variables in this study. The ASCUS, LSIL, HSIL and CIN1 grades 1, 2 and 3 were registered as index incident cases for the statistical analysis. SIL and CIN cases were combined to form a general cervical atypia category. Cervical atypia findings were registered by the interval between menarche and FSI or the start of OC use to form four mutually exclusive different individual outcome variables: (1) cervical atypia with a shorter than 3-year lag between menarche and FSI, (2) cervical atypia with an equal to or longer than 3-year lag between menarche and FSI, (3) cervical atypia with a shorter than 3-year lag between menarche and OC use and (4) cervical atypia with an equal to or longer than 3-year lag between menarche and OC use.

Univariate and multivariable logistic regression models were used to calculate the risk, reported as ORs with 95% CI. The statistical analysis was performed using Stata V.14.0 (Stata Corp LP, Statistical Software: Release 14).

4.6.4 Paper IV

The main independent variables of the study were *C. trachomatis* status, HPV16/18 status, and duration of OC use. Information about OC use was available from the questionnaires. The endpoint was squamous intraepithelial lesions (SIL) divided into low-grade and high-grade squamous intraepithelial lesions (LSIL and HSIL, respectively).

Odds ratios (OR) with 95% confidence intervals (CI) were calculated using logistic regression models to assess the risk of SIL associated with *C. trachomatis* infection at age 18.5 and 22 years. The risk estimates were adjusted to account for the potential confounding due to HPV DNA, condom use, number of sexual partners and smoking using multivariable logistic regression. Finally, the joint effect of duration of OC use and *C. trachomatis* was calculated. All analyses were done using Stata version 14.0 (Stata Corp LP, Statistical Software: Release 14, College Station, TX, USA).

5 SUMMARY OF RESULTS

5.1 Paper I

Altogether, there were 52 HPV16-seropositive and 245 HPV16-seronegative women, and 23 HPV18-seropositive and 263 HPV18-seronegative women who had donated first trimester serum samples to the Finnish Maternity Cohort and participated in a study on the relationship between OC use and the risk of breast cancer. For 11 samples, the HPV18 serology data was missing due to inadequate sample volume. Demographic and key study characteristics showed no major differences between HPV16- and/or HPV18-seropositive and seronegative women. The OC use distributions, however, differed between the HPV16- and/or HPV18-seropositives and the seronegatives. The proportion of women with long-term OC use was roughly two times higher in the HPV16/18-seropositive women as compared to the seronegatives.

There appeared to be an increased risk (OR 2.9, 95% CI: 0.9–9.1) of HPV16/18 seropositivity among women with prolonged (5 or more years) OC use. Those who had used OCs for less than 5 years had approximately the same risk of HPV16/18 seropositivity as non-users.

5.2 Paper II

A total of 914 women who met the inclusion criteria, were eligible for this study. The mean age at sexual debut was 15.9 (12–22) years. The total numbers of OC users and never-users were 843 and 62, respectively. Some data on the time since the start of OC use was missing, which decreased the number of OC users in the analysis to 821 while categorising them further by time of OC use.

The incidence rates of LSIL (per 100 person-years) were somewhat higher among never users 6.9 (95% CI: 4.2–11.4) or the women who had just started the use of OCs (0 to 1 year ago), 7.7 (95% CI: 2.5–23.9) as compared to those who had started more than a year ago 5.6 (95% CI: 4.8–6.5) (Table 1). Similarly, the incidence rates of CIN1 per 100 person-years appeared to be higher among never users (2.6, 95% CI: 1.1–5.7) or women who had just started the use of OC (2.3, 95% CI: 0.3–16.3) compared to women who had started to use OC for more than a year ago (1.0, 95% CI: 0.7–1.5).

Overall, there was no significantly increased risk of ASCUS associated with OC use (Table 2). On the contrary, the adjusted relative risk of CIN1 in women who had started the use of OCs more than a year ago was significantly reduced (OR 0.2, 95% CI: 0.1–0.7) as compared to never users (Table 2).

Table 1. Incidence rate (per 100 women years) of abnormal cytological findings by time since the start of oral contraceptive (OC) use during a clinical follow-up of 4 years

Category	OC-use 0-1 years (N=11)		OC-use 2-3 years (N=52)		OC-use 4-5 years (N=192)		OC-use 6-7 years (N=399)		OC- use ≥ 8 years (N=167)		OC-use >1 year (N=810)		Never-users (N=62)	
	n	Incidence rate (95% CI)	n	Incidence rate (95% CI)	n	Incidence rate (95% CI)	n	Incidence rate (95% CI)	n	Incidence rate (95% CI)	n	Incidence rate (95% CI)	n	Incidence rate (95% CI)
ASCUS	1	2.4 (0.3-17.1)	11	5.6 (3.1-10.1)	29	4.0 (2.8-5.8)	67	4.6 (3.6-5.8)	34	5.6 (4.0-7.8)	141	4.7 (4.0-5.6)	9	4.0 (2.1-7.7)
LSIL	3	7.7 (2.5-23.9)	11	5.8 (3.2-10.5)	40	5.7 (4.2-7.8)	78	5.4 (4.3-6.7)	35	5.7 (4.1-8.0)	164	5.6 (4.8-6.5)	15	6.9 (4.2-11.4)
HSIL	0	NA	0	NA	0	NA	4	0.3 (0.1-0.7)	0	NA	4	0.1 (0.0-0.3)	1	0.4 (0.1-3.0)
Any abnormality*	4	9.6 (3.6-25.6)	16	8.2 (5.0-13.3)	57	7.9 (6.1-10.2)	125	8.5 (7.2-10.2)	54	8.8 (6.8-11.5)	252	8.4 (7.4-9.5)	22	9.8 (6.4-14.9)

*Any abnormality means either ASCUS or LSIL or HSIL. Table adapted from paper II

Table 2. Adjusted relative risk (odds ratio, OR with 95% confidence interval, 95% CI) of abnormal cytological and histopathological findings associated with time since the start of oral contraceptive (OC) use

Category	OC-use 0-1 years (N=11)			OC- use >1 year (N=810)			Never users (N=62)	
	n	Crude OR	Adj. OR (95% CI)	n	Crude OR	Adj. OR (95% CI)	n	OR
ASCUS	1	0.5	0.5 (0.1-4.6)	141	1.2	1.0 (0.4-2.3)	9	1.0
LSIL	3	1.1	1.0 (0.2-4.6)	164	0.7	0.6 (0.3-1.3)	15	1.0
CIN1	1	0.9	0.7 (0.1-7.3)	33	0.3	0.2 (0.1-0.7)	6	1.0
CIN1+	1	0.7	0.7 (0.1-6.9)	47	0.4	0.4 (0.1-1.1)	7	1.0

Table adapted from paper II

5.3 Paper III

Almost one-third of the women with cervical atypia (55 of 197, 28.0%) had more than 10 lifetime sexual partners. Half of the women with or without cervical atypia, 49.2% and 45.4% respectively, did not regularly use condoms. The vast majority of the women (179 of 197, 90.9%) with cervical atypia had used OCs. Age at the start of OC use for the majority of these women (104 of 197, 52.8%) was between 12 and 16 years.

During the 4-year follow-up, 201 women (22%) tested positive for HPV16, and 120 (13.1%) tested positive for HPV18. One-third of women with either HPV16 or HPV18 or both were diagnosed with cervical atypia during the follow-up. The number of women who tested positive for *C. trachomatis* was 102 (11.2%), and the number of *C. trachomatis*-positive women with cervical atypia was 22 (11.2%). Out of 913 women, 156 (17.1%) had ASCUS, 189 (20.7%) had LSIL, 5 (0.6%) had HSIL, 40 (4.4%) had CIN1, 22 (2.41%) had CIN2 and 8 (0.9%) had CIN3. One hundred ninety-seven (21.6%) of the 913 women were identified with cervical atypia.

Cervical atypia risk estimates associated with HPV 16/18 were increased (OR: 1.8, 95% CI: 1.1–2.7; and OR: 1.4, 95% CI: 1.0–2.1) in the longer (≥3 years) interval categories (between menarche and first sexual intercourse and between menarche and the start of OC use, respectively). Also, the cervical atypia risk associated with *C. trachomatis* was increased (OR: 1.8, 95% CI: 1.0–3.6; and OR 2.2, 95% CI: 1.0–5.1), however, in the short (<3 years) interval categories (Table 3).

Table 3. Relative risk (odds ratio, OR, with 95% confidence interval, CI) of cervical atypia (cytological squamous intraepithelial lesion, SIL and/or cervical intraepithelial neoplasia grade 1 or worse, CIN1+) associated with different co-variables in analyses stratified by the interval between menarche (M) and age at first sexual intercourse (FSI, Category 1), or between menarche and the age at start of oral contraceptive (OC, Category 2) use in young adult women followed up for 4 years

	Frequency and relative risk of cytological atypia: SIL or CIN1 in the different categories by interval from menarche							
	Category 1				Category 2			
	Menarche to FSI <3 y		Menarche to FSI ≥3 y		Menarche to start of OCs <3 y		Menarche to start of OCs ≥3 y	
	SIL/CIN1+		SIL/CIN1+		SIL/CIN1+		SIL/CIN1+	
Variable	n/N	OR (95%CI)	n/N	OR (95%CI)	n/N	OR (95%CI)	n/N	OR (95%CI)
HPV 16/18								
Pos.	21/115	1.0 (0.6-2.0)	45/144	1.8 (1.1-2.7)	13/69	1.4 (0.6-3.0)	53/190	1.4 (1.0-2.1)
Neg.	33/186	1	87/422	1	18/123	1	102/484	1
Chlamydia								
Pos.	14/54	1.8 (1.0-3.6)	8/44	0.7 (0.3-1.6)	10/39	2.2 (1.0-5.1)	12/59	0.8 (0.4-1.6)
Neg.	40/248	1	124/522	1	21/153	1	143/616	1
Smoking								

Yes	34/165	1.5 (0.8-2.7)	52/209	1.1 (0.8-1.7)	22/108	2.1 (1.0-5.0)	64/266	1.1 (0.8-1.6)
No	20/134	1	80/356	1	9/84	1	91/405	1
Condom use								
Yes	22/146	0.7 (0.4-1.2)	59/256	0.9 (0.6-1.3)	15/102	0.6 (0.3-1.3)	66/300	0.9 (0.6-1.3)
No	29/139	1	61/251	1	16/80	1	74/309	1
LNSP								
High	39/215	1.1 (0.6-2.0)	64/245	1.3 (0.9-2.0)	24/135	1.5 (0.6-3.8)	79/325	1.2 (0.8-1.7)
Low	15/87	1	68/321	1	7/57	1	76/350	1

*** LNSP=** lifetime number of sexual partners, high= 5 or more. Table adapted from paper III

In univariate analyses, the risk of cervical atypia associated with the short interval between menarche and age at the start of OC use appeared to be somewhat decreased (OR: 0.7, 95% CI 0.4–1.3) when the interval between menarche and age at first sexual intercourse was short (Table 4). With the long-term interval between menarche and the start of OC use, the lag between menarche and FSI had no material effect on the atypia risk (Table 4). When we performed multivariable analyses with stepwise exclusion of one variable at a time from the multivariable model (smoking, condom use, *C. trachomatis*, HPV 16/18 infection) to check the interdependency of the interval between menarche and age at the start of OC use or between menarche and age at first sexual intercourse, the association remained insignificant (data not shown).

Table 4. Relative risk (odds ratio, OR, with 95% confidence interval, CI) of cervical atypia (cytological squamous intraepithelial lesion, SIL and/or cervical intraepithelial neoplasia grade 1 or worse, CIN1+) stratified by the interval between menarche (M) and age at first sexual intercourse (FSI) or between menarche and the age at start of oral contraceptive (OC) use in young adult women followed up for 4 years

Frequency and relative risk of cytological atypia: SIL or CIN1 in the different categories by interval from menarche

	Category 1			**Category 2**		
	Menarche to FSI <3 y			**Menarche to start of OCs ≥3y**		
	SIL/CIN1+			**SIL/CIN1+**		
Variable	**n/N**	**OR (95%CI)**		**n/N**	**OR (95%CI)**	
Lag between FSI and menarche						
<3 yrs.	53/301	NA		23/110	0.9 (0.5-1.4)	0.9 (0.8-1.0)
≥3 yrs	NA	NA		132/565	1	*Interval (cont.)
Lag between start of OCs and menarche						
<3 yrs.	30/191	0.7 (0.4-1.3)	0.9 (0.9-1.0)	NA	NA	
≥3 yrs	23/110	1	*Interval (cont.)	155/675	NA	

*Interval as continuous variable. Table adapted from paper III

5.4 Paper IV

At the first visit (age 18.5 years), the total number of SIL cases was 940. The age at first sexual intercourse, number of new sexual partners and OC use were materially similar in the SIL cases and healthy controls at the first visit. There were no notable differences in HPV16/18 positivity either. At the second visit, the total number of SIL cases was 129 (1.9%) of the 6618 study participants. The number of high-grade SIL (HSIL) cases was 27; women with SIL had 5 or more sexual partners twice as often as healthy women with normal cytology. OC use was comparable between SIL cases and the healthy controls.

The risk of SIL associated with *C. trachomatis* was not materially increased at the first visit. When the potential confounders were adjusted, a unit risk prevailed.

At the second visit (22 years of age), the risk of SIL was highly significantly increased (OR 4.6) in *C. trachomatis*–positive women as compared to *C. trachomatis*–negative women. The odds ratios remained significantly higher (OR: 4.3, CI: 2.2–8.5) even after adjusting for HPV16/18, condom use, number of sexual partners and smoking (Table 5).

Table 5. Risk of cervical cytological squamous intraepithelial neoplasia by C. trachomatis at 18.5 and 22 years old

		SIL		Adjusted SIL*
Category	**N**	**n**	**OR (95% CI)**	**OR (95% CI)**
At 18.5 years				
Chlamydia negative women	10512	901	1	1
Chlamydia positive women	408	39	1.1 (0.8-1.6)	1.0 (0.6-1.5)
At 22 years				
Chlamydia negative women	5352	86	1	1
Chlamydia positive women	198	14	4.6 (2.6-8.3)	4.3 (2.2-8.5)

Note: N is the number of C. trachomatis in each age group. *Adjusted for HPV16/18, condom use last year, smoking and no. of sexual partners. Adapted from paper IV

Finally, the joint effect of *C. trachomatis* and the duration of OC use was assessed. Among the 18.5-year-olds, no joint effect between *C. trachomatis* and the duration of OC use was observed (Table 6). On the contrary, the adjusted risk of SIL was almost four-fold (OR: 4.7, CI: 1.7–12.8) in the 22-year-olds *C. trachomatis*–positive women who had used OC for 5 or more years compared to C. *trachomatis*–negative short-term OC users (Table 7). The risk was adjusted for HPV16/18, condom use, number of sexual partners and smoking. Under a multiplicative model, the expected joint effect of *C. trachomatis* positivity and 5 or more years of OC use had an odds ratio of 2.9 and a confidence interval of 0.6-14.0. The observed joint effect of 4.7 was 1.6 times higher than expected on a multiplicative scale. Under an additive scale, the relative excess risk from interaction (RERI) had an odds ratio of 1.8 and a confidence interval of -3.5-7.2.

Table 6. Risk of cervical squamous intraepithelial neoplasia (SIL) by joint effect of duration of oral contraceptive (OC) use and C. trachomatis positivity at 18.5 years

		SIL		Adjusted SIL*
Risk Factors	**N**	**n**	**OR (95% CI)**	**OR (95% CI)**
CT# Duration of OC use				
0 0	2746	233	1	1
0 1	3675	298	0.9 (0.8-1.1)	0.9 (0.7-1.1)
1 0	88	7	0.9 (0.4-1.9)	0.7 (0.3-1.7)
1 1	126	11	1.0 (0.5-1.8)	0.9 (0.4-1.7)

CT= C. trachomatis. *Adjusted for HPV16/18, condom use last year, smoking and no. of sexual partners. Adapted from paper IV

Table 7. Risk of cervical squamous intraepithelial neoplasia (SIL) by joint effect of duration of oral contraceptive (OC) use and C. trachomatis positivity at 22 years

		SIL		Adjusted SIL*
Risk Factors	**N**	**n**	**OR (95% CI)**	**OR (95% CI)**
CT# Duration of OC use				
0 0	2293	30	1	1
0 1	1958	36	1.4 (0.9-2.3)	1.0 (0.5-1.9)
1 0	79	3	3.0 (0.9-9.9)	2.9 (0.8-10.3)
1 1	90	8	7.3 (3.3-16.6)	4.7 (1.7-12.8)

CT= C. trachomatis. *Adjusted for HPV16/18, condom use last year, smoking and no. of sexual partners. Adapted from paper IV

6 DISCUSSION

6.1 Oral contraceptives and cervical atypia

We evaluated the association between OC use and cervical cytological and histological cellular abnormalities. We found a slightly decreased risk of CIN1 in women who had used OC for more than a year. Our finding that OC use is not associated with cervical neoplasia is comparable to a population-based study among Latin American screening participants (Longatto-Filho et al., 2011). This study found no risk of either high-grade cervical intraepithelial lesions or hrHPV infection associated with OC use. Our study finding that OC use might be protective against CIN1 is comparable to a study conducted in Australia which assessed the risk of cervical intraepithelial lesions associated with condom and OC use. The study found that prolonged OC use, not the use of condoms, could be protective in reducing the risk of CIN (Hui et al., 2014).

We did not find an increased risk of cytological abnormality associated with OC use, which is in line with the earlier studies (Binesh et al., 2013; Giuliano et al., 2004; Kazerooni & Mosalaee, 2002) and also suggests that misclassification of cytological abnormality may be an issue. In a study by Kruger-Kjaer et al., long-term OC use was associated with the development of HSIL corresponding to CIN2/3 but not with ASCUS and LSIL (Krüger-Kjær et al., 1998). In our study, the lack of a significant positive or negative association between cytological abnormality and OC use might be attributed to the low number of abnormal cytological cases. Also, the trial participants were quite young (16 to 22 years) during the study period, so there was a relatively short time for the development of high-grade cervical lesions.

We found cervical atypia was not associated with an early start of sexual activity after menarche. There was no risk of cervical atypia associated with the short interval between menarche and OC use and/or first sexual intercourse. This finding is contradictory to the finding of Ruiz et al., who first reported that a short interval between menarche and age at the FSI is a predictor of cervical cytological abnormalities and CIN (Ruiz et al., 2012). While our homogeneous study population had ample power to detect a threefold increased risk, their study population was heterogeneous and had only baseline questionnaire data on sexual risk-taking behaviour, which could not elaborate possible changes in the risk-taking behaviour during the follow-up.

6.2 HPV, Chlamydia, oral contraceptives, and cervical atypia

The risk of HPV 16/18 seropositivity increased with the increase in the duration of OC use. This non-significantly increased risk did not change much after adjusting for age, smoking and *C. trachomatis*. This concurs with Rousseau et al., who examined the risk factors for non-oncogenic, oncogenic and HPV16 cervical infections. The women who developed infections with exclusively non-oncogenic types and any HPV16 were compared with the women who were HPV-negative throughout the follow-up period of 1 year. OC use was strongly and exclusively associated with the risk of hrHPV and HPV16 infections (Rousseau et al., 2000). OC use has also been found to promote the persistence of oncogenic HPV infection in a population-based prospective cohort study examining the risk factors for the persistence of high-risk HPV infections among Danish women (Nielsen et al., 2010).

We found that the risk of cervical atypia associated with HPV16/18 infection was increased with a more-than-3-year interval between menarche and first sexual activity. There was no risk associated with HPV16/18 infection when there was a short interval between menarche and first sexual intercourse. Our finding is in line

with Collins et al., who found that the cervical HPV infection increases with the interval between menarche and first sexual intercourse (Collins et al., 2005). At the same time, our finding contradicts the studies which suggest that a short interval between menarche and first intercourse increases the risk of cervical HPV infection (Kahn et al., 2002; Shew et al., 1994).

We also found that the *C. trachomatis*–associated risk of cervical atypia was increased with a short interval between menarche and first sexual intercourse as well as between menarche and the start of OC use. Our group has earlier reported that when *C. trachomatis* infection precedes or cooccurs with HPV infection the risk of high-grade cervical neoplasia associated with the joint infection is very high (Luostarinen et al., 2004). Similarly, another study has found that concomitant HPV persistence and *C. trachomatis* infections at a young age are likely to increase the risk of cervical neoplasia (Jensen et al., 2014). Increased risk of squamous cell cervical cancer associated with *C. trachomatis* infection among HPV-positive women has been reported (Smith et al., 2002). *C. trachomatis* may not only be a marker of sexual risk-taking behaviour but also facilitate HPV persistence, which ultimately increases the risk of neoplastic lesions (Paavonen, 2001). Furthermore, *C. trachomatis* has also been proven to set the stage for cervical carcinogenesis, leading to CIN3, possibly even, independently of HPV (Lehtinen et al., 2011; Wallin et al., 2002).

The *C. trachomatis*–associated risk of SIL was high among 22- but not 18.5-year-old women. This study found that the joint effect of *C. trachomatis* and long-term duration of OC use (more than 5 years) was more than fourfold even after adjusting for the possible confounders among 22-year-old women. The observed joint effect was higher than expected on both multiplicative and additive scales among 22-year-old women. While OCs are one of the most common contraceptive methods among adolescents, they provide protection against unwanted pregnancies but not against sexually transmitted infections (Halvarsson et al., 2012). The biological plausibility

of assuming a synergistic interaction between OC use and cervical *C. trachomatis* infection is that the use of OCs may increase the growth and persistence of *C. trachomatis* infection by altering the immune response, especially among those who do not use a barrier method of contraception (Baeten et al., 2001). Cottingham and Hunter published a study about the association between *C. trachomatis* and OC use. They found a two-fold risk of increased *C. trachomatis* infection among OC users (Cottingham & Hunter, 1992). Another observation similar to our study finding was made by Baeten et al. in a study which found an increased risk of *C. trachomatis* infection among OC users compared to other STDs (Baeten et al., 2001). Our joint effect risk of *C. trachomatis* and prolonged OC use is consistent with these studies.

6.3 Strengths and limitations of the study

One strength of our study is that we obtained the OC information from the FSHS cohort, which is relatively complete information over time on OC use. The identification of archived serum samples from the FMC serum bank Oulu for the determination of HPV16/18 VLP ELISA measures antibodies in Paper I is another strength of our study.

Next, the data used in our studies stem from a large cohort (PATRICIA), with meticulous clinical follow-up over 4 years, and the standardised clinical and laboratory procedures are noteworthy. Over the entire follow-up period, the trial participants received regular sexual health counselling, which probably helped in retaining the participants and reduced possible confounding and bias in our study. Cervical cytological sampling was done every 6 months irrespective of OC use. The questionnaire-based information about the sexual risk-taking characteristics of the study subjects over time was comprehensive. Moreover, questionnaire-based information regarding sexual behaviour is supposed to have adequate validity and reliability (Brener et al., 1995; Kahn et al., 2001).

The longitudinal study design in Paper IV allowed the evaluation over time of OC use and timely measurement (and treatment) of *C. trachomatis* infection. Another strength is the well-controlled and sensitive testing of *C. trachomatis* by *PCR*.

The first limitation of our study is that in Paper I, we had a small study size. It is not adequate for stratified or interaction analyses, which otherwise would have elaborated the relative interdependency of various risk factors of HPV16/18 seropositivity (Lehtinen et al., 2011). Thus, no inferences could be made as to the action mechanisms of prolonged OC use on the acquisition of serologically indicated persistent HPV16/18 infection.

Next, the questionnaires were filled in at the end of the clinical trial, and random breaks in OC use were not registered. The study questionnaires used were self-

reported at the ages of 18 and 22 years, the latter of which is subject to recall bias. The study participants were distributed free contraceptives during the trial period, which might have increased the proportions of OC and condom users in our study. The use of the overall cervical atypia endpoint, which was necessary to retain the statistical power of the study strata, could be another limitation of our study.

The number of HSIL findings is small in Study IV. Furthermore, inadequate information on the use of barrier methods of contraception did not allow control of possibly associated confounding results. We have not considered the missing values in our study, as they did not vary the result while putting them into the multivariable model. Finally, the lack of information about the types of OC pills used might have affected the risk, as different types of OCs have different hormonal compositions.

6.4 Summary and conclusions

The main findings and conclusions of the presented work are as follows:

Prolonged OC use is associated with HPV16/18 seropositivity and possibly associated with persistent HPV16/18 infection.

However, the use of OCs did not increase the risk of cervical atypia. Instead, established use of OCs might be protective against mild cervical abnormality and CIN1, that is, the clinical manifestations of cervical HPV infection. Larger prospective studies aiming to determine the association between cervical lesions and OCs are required.

Our study does not support the hypothesis that a short interval between menarche and age at the start of sexual activity always increases the risk of cervical atypia. However, acquiring *C. trachomatis* infections at an early age may set the stage for cervical carcinogenesis, and the infection should be identified and treated.

C. *trachomatis*–associated atypia/SIL risk was much higher if the women had used OCs for more than 5 years. People who use OCs as a means of contraception may not use barrier methods of contraception and hence are prone to many sexually transmitted infections. Thus, condom use should be enforced among women using oral/hormonal contraceptives.

6.5 References

1. American Cancer Society. (2021). What is cervical cancer? Retrieved from https://www.cancer.org/cancer/cervical-cancer/about/what-is-cervical-cancer.html
2. Anttila, T., Saikku, P., Koskela, P., Bloigu, A., Dillner, J., Ikäheimo, I…….Paavonen, J. (2001). Serotypes of Chlamydia trachomatis and Risk for Development of Cervical Squamous Cell Carcinoma. *JAMA, 285*(1), 47-51. doi:10.1001/jama.285.1.47
3. Appleby, P., Beral, V., de Gonzalez, A. B., Colin, D., Franceschi, S., Goodhill, A….Sweetland, S. (2007). Cervical cancer and hormonal contraceptives: collaborative reanalysis of individual data for 16 573 women with cervical cancer and 35 509 women without cervical cancer from 24 epidemiological studies. *Lancet, 370*(9599), 1609-1621. doi:10.1016/S0140-6736(07)61684-5
4. Arbyn, M., Weiderpass, E., Bruni, L., de Sanjosé, S., Saraiya, M., Ferlay, J., & Bray, F. (2020). Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. *Lancet, 8*(2), e191-e203. doi:10.1016/S2214-109X(19)30482-6
5. Baeten, J. M., Nyange, P. M., Richardson, B. A., Lavreys, L., Chohan, B., Martin, H. L…….Kreiss, J. K. (2001). Hormonal contraception and risk of sexually transmitted disease acquisition: Results from a prospective study. *Am J Obstet Gynecol, 185*(2), 380-385. doi:10.1067/mob.2001.115862
6. Baird, D. T., & Glasier, A. F. (1993). Hormonal Contraception. *N Engl J Med, 328*(21), 1543-1549. doi:10.1056/NEJM199305273282108
7. Becker, T. M., Wheeler, C. M., McGough, N. S., Parmenter, C. A., Stidley, C. A., Jamison, S. F., & Jordan, S. W. (1994). Cigarette smoking and other risk factors for cervical dysplasia in southwestern Hispanic and non-Hispanic white women. *Cancer Epidemiol Biomarkers Prev, 3*(2), 113-119.
8. Beral, V. (2015). Reprint of "Cancer of the cervix: A sexually transmitted infection?". *Cancer Epidemiol, 39*(6), 1148-1151. doi:10.1016/j.canep.2015.08.005
9. Binesh, F., Akhavan, A., Pirdehghan, A., & Davoodi, M. (2013). Does oral contraceptive pill increase the risk of abnormal Pap smear? *Iran J Reprod Med, 11*(9), 761-766.
10. Bosch, F. X., Manos, M. M., Munoz, N., Sherman, M., Jansen, A. M., Peto, J…..Shan, K. V. (1995). Prevalence of Human Papillomavirus in Cervical Cancer: a Worldwide Perspective. *JNCI, 87*(11), 796-802. doi:10.1093/jnci/87.11.796
11. Boshart, M., Gissmann, L., Ikenberg, H., Kleinheinz, A., Scheurlen, W., & Hausen, H. (1984). A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. *EMBO J, 3*(5), 1151-1157. doi:10.1002/j.1460-2075.1984.tb01944.x
12. Boshart, M., Weber, F., Jahn, G., Dorsch-H⇒ler, K., Fleckenstein, B., & Schaffner, W. (1985). A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell, 41*(2), 521-530. doi:10.1016/S0092-8674(85)80025-8
13. Brener, N. D., Collins, J. L., Kann, L., Warren, C. W., & Williams, B. I. (1995). Reliability of the Youth Risk Behavior Survey Questionnaire. *Am J Epidemiol, 141*(6), 575-580. doi:10.1093/oxfordjournals.aje.a117473

14. Brinton, L. A., Tashima, K. T., Lehman, H. F., Levine, R. S., Mallin, K., Savitz, D. A…..Fraumeni, J. F. J. (1987). Epidemiology of cervical cancer by cell type. *Cancer Res, 47*(6), 1706-1711.
15. Collins, S. I., Mazloomzadeh, S., Winter, H., Rollason, T. P., Blomfield, P., Young, L. S., & Woodman, C. B. J. (2005). Proximity of first intercourse to menarche and the risk of human papillomavirus infection: A longitudinal study. *Int J Cancer, 114*(3), 498-500. doi:10.1002/ijc.20732
16. Collins, S., Rollason, T. P., Young, L. S., & Woodman, C. B. J. (2009). Cigarette smoking is an independent risk factor for cervical intraepithelial neoplasia in young women: A longitudinal study. *Eur J Cancer, 46*(2), 405-411. doi:10.1016/j.ejca.2009.09.015
17. Cooper DB, Mahdy H. (2021). Oral Contraceptive Pills. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK430882/
18. Cottingham, J., & Hunter, D. (1992). Chlamydia trachomatis and oral contraceptive use: a quantitative review. *Genitourin Med, 68*(4), 209-216. doi:10.1136/sti.68.4.209
19. Cromwell, P. F., & Daley, A. M. (2000). Oral contraceptive pills: Considerations for the adolescent patient. *Journal of Pediatric Health Care, 14*(5), 228-234. doi:10.1067/mph.2000.106001
20. Daniel Deluca, G., Basiletti, J., Schelover, E., Díaz Vásquez, N., Mario Alonso, J., Marcelo Marín, H….Alejandra Picconi, M. (2011). Chlamydia trachomatis as a probable cofactor in human papillomavirus infection in aboriginal women from northeastern Argentina. *Braz J Infect Dis, 15*(6), 567-572. doi:10.1016/S1413-8670(11)70252-5
21. Deacon, J. M., Evans, C. D., Yule, R., Desai, M., Binns, W., Taylor, C., & Peto, J. (2000). Sexual behaviour and smoking as determinants of cervical HPV infection and of CIN3 among those infected: a case-control study nested within the Manchester cohort. *Br J Cancer, 83*(11), 1565-1572. doi:10.1054/bjoc.2000.1523
22. Delgado-Rodriguez, M., Sillero-Arenas, M., Martin-Moreno, J., & Galvez-Vargas, R. (1992). Oral contraceptives and cancer of the cervix uteri: A meta-analysis. *Acta Obstet Gynecol Scand, 71*(5), 368-376. doi:10.3109/00016349209021075
23. Dillner, J., Lehtinen, M., Sapp, M., Schiller, J. T., Hakulinen, T., Thoresen, S…..Paavonen, J. (1997). Prospective seroepidemiologic study of human papillomavirus infection as a risk factor for invasive cervical cancer. *JNCI, 89*(17), 1293-1299. doi:10.1093/jnci/89.17.1293
24. Dillner, J., Andersson-Ellström, A., Hagmar, B., & Schiller, J. (1999). High risk genital papillomavirus infections are not spread vertically. *Reviews in Medical Virology, 9*(1), 23-29. doi:10.1002/(SICI)1099-1654(199901/03)9:13.0.CO;2-S
25. Dillner, J., Kallings, I., Brihmer, C., Bo Sikström, Koskela, P., Lehtinen, M….Per Anders Mårdh. (1996). Seropositivities to Human Papillomavirus Types 16, 18, or 33 Capsids and to Chlamydia trachomatis Are Markers of Sexual Behavior. *J Infect Dis, 173*(6), 1394-1398. doi:10.1093/infdis/173.6.1394
26. Durst, M., Gissmann, L., Ikenberg, H., & Harald, Z. H. (1983). A Papillomavirus DNA from a Cervical Carcinoma and Its Prevalence in Cancer Biopsy Samples from Different Geographic Regions. *Proc Natl Acad Sci U S A, 80*(12), 3812-3815. doi:10.1073/pnas.80.12.3812

27. Ferlay, J., Ervik, M., Lam, F., Colombet, M., Mery, L., Piñeros, M……..Bray, F. (2020). Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today, [Accessed 16th August 2022]
28. Ferrera, A., Baay, M. F. D., Herbrink, P., Figueroa, M., Velema, J. P., & Melchers, W. J. G. (1997). A sero-epidemiological study of the relationship between sexually transmitted agents and cervical cancer in Honduras. *Int J Cancer, 73*(6), 781-785. doi:10.1002/(SICI)1097-0215(19971210)73:63.0.CO;2-Z
29. Finnish Student Health Service. (n.d.). Retrieved from https://www.yths.fi/en/fshs/finnish-student-health-service/organisation-en/
30. Finnish Institute for Health and Welfare (2022). Prevalence of chlamydia in Finland. Retrieved from https://thl.fi/fi/web/infektiotaudit-ja-rokotukset/taudit-ja-torjunta/taudit-ja-taudinaiheuttajat-a-o/klamydia/klamydian-esiintyvyys-suomessa
31. Fonseca-Moutinho, J. (2011). Smoking and Cervical Cancer. *ISRN Obstet Gynecol,* 847684-6. doi:10.5402/2011/847684
32. Forman, D., de Martel, C., Lacey, C. J., Soerjomataram, I., Lortet-Tieulent, J., Bruni, L., . . . Franceschi, S. (2012). Global burden of human papillomavirus and related diseases. *Vaccine, 30*, F12-F23. doi:10.1016/j.vaccine.2012.07.055
33. Franceschi, S., & Vaccarella, S. (2015). Beral's 1974 paper: A step towards universal prevention of cervical cancer. *Cancer Epidemiol, 39*(6), 1152-1156. doi:10.1016/j.canep.2015.10.019
34. Frega, A., Scardamaglia, P., Piazze, J., Cerekja, A., Pacchiarotti, A., Verrico, M., & Moscarini, M. (2007). Oral contraceptives and clinical recurrence of human papillomavirus lesions and cervical intraepithelial neoplasia following treatment. *Int J Gynaecol Obstet, 100*(2), 175-178. doi:10.1016/j.ijgo.2007.08.023
35. Gagnon, F. (1950). Contribution to the study of the etiology and prevention of cancer of the cervix of the uterus. *Am J Obstet Gynecol, 60*(3), 516-522. doi:10.1016/0002-9378(50)90422-4
36. Giuliano, A. R., Papenfuss, M., Mendez Brown de Galaz, Elena, Feng, J., Abrahamsen, M., Denman, C., Guernsey de Zapien, J……Hatch, K. (2004). Risk factors for squamous intraepithelial lesions (SIL) of the cervix among women residing at the US-Mexico border. *Int J Cancer, 109*(1), 112-118. doi:10.1002/ijc.11656
37. Halvarsson, V., Strom, S., & Liljeros, F. (2012). The prescription of oral contraceptives and its relation to the incidence of chlamydia and abortion in Sweden 1997—2005. *Scand J Public Health, 40*(1), 85-91. doi:10.1177/1403494811421977
38. Handsfield, H. H., Jasman, L. L., Roberts, P. L., Hanson, V. W., Kothenbeutel, R. L., & Stamm, W. E. (1986). Criteria for Selective Screening for Chlamydia trachomatis Infection in Women Attending Family Planning Clinics. *JAMA, 255*(13), 1730-1734. doi:10.1001/jama.1986.03370130086029
39. Hannaford, P. C., Selvaraj, S., Elliott, A. M., Angus, V., Iversen, L., & Lee, A. J. (2007). Cancer risk among users of oral contraceptives: cohort data from the Royal College of General Practitioner's oral contraception study. *Bmj, 335*(7621), 651-654. doi:10.1136/bmj.39289.649410.55

40. Hassani, K. F., Kosunen, E., & Rimpelä, A. (2006). The Use of Oral Contraceptives Among Finnish Teenagers from 1981 to 2003. *J Adolesc Health, 39*(5), 649-655. doi:10.1016/j.jadohealth.2006.05.022
41. Hassani, K. F. (2010). Changes in sexual behavior and hormonal contraceptives use among finnish adolescents (doctoral dissertation, University of Tampere, Finland). Retrieved from https://trepo.tuni.fi/handle/10024/66630
42. Hausen, H. Z., Schulte-Holthausen, H., Wolf, H., Dörries, K., & Egger, H. (1974). Attempts to detect virus-specific DNA in human tumors. II. Nucleic acid hybridizations with complementary RNA of human herpes group viruses. *Int J Cancer, 13*(5), 657-664. doi:10.1002/ijc.2910130510
43. Hemminki, E., Luostarinen, T., Pukkala, E., Apter, D., & Hakulinen, T. (2002). Oral contraceptive use before first birth and risk of breast cancer: a case control study. *BMC Womens Health, 2*(1), 9. doi:10.1186/1472-6874-2-9
44. Hildesheim, A., Herrero, R., Helgesen, K., Alfaro, M., Hutchinson, M., Balmaceda, I…..Rodriguez, A. C. (2001). HPV co-factors related to the development of cervical cancer: results from a population-based study in Costa Rica. *Br J Cancer, 84*(9), 1219-1226. doi:10.1054/bjoc.2001.1779
45. Hui, J. C., Lee, A. H., Colville, L., Xu, D., & Binns, C. W. (2014). Original Article: Condom and oral contraceptive use and risk of cervical intraepithelial neoplasia in Australian women. *Journal of Gynecologic Oncology (JGO), 25*(3), 183.
46. International Agency for Research on Cancer. (2021). *Colposcopy and treatment of cervical intraepithelial neoplasia: a beginners' manual, Edited by J.W. Sellors and R. Sankaranarayanan.* Retrieved from https://screening.iarc.fr/colpochap.php?chap=2 [Accessed 10th December 2021]
47. International Agency for Research on Cancer. (2003). *Cervical cancer prevention factsheet*. Retrieved from https://screening.iarc.fr/doc/RH_natural_history_of_cc_fs.pdf [Accessed 10th December 2021]
48. International Agency for Research on Cancer. (2020). *GLOBOCAN 2020, Cervix uteri.* Retrieved from https://gco.iarc.fr/today/data/factsheets/cancers/23-Cervix-uteri-fact-sheet.pdf [Accessed 10th December 2021]
49. Jensen, K. E., Thomsen, L. T., Schmiedel, S., Frederiksen, K., Norrild, B., van den Brule, A….Kjær, S.,K. (2014). Chlamydia trachomatis and risk of cervical intraepithelial neoplasia grade 3 or worse in women with persistent human papillomavirus infection: a cohort study. *Sex Transm Infect, 90*(7), 550-555. doi:10.1136/sextrans-2013-051431
50. Kahn, J., Goodman, E., Kaplowitz, R., Slap, G., & Emans, S. (2001). Validity of Adolescent and Young Adult Self-Report of Papanicolaou Smear Results. *J Low Genit Tract Dis, 5*(2), 115. doi:10.1046/j.1526-0976.2001.52011-10.x
51. Kahn, J. A., Rosenthal, S. L., Succop, P. A., Ho, G. Y. F., & Burk, R. D. (2002). The interval between menarche and age of first sexual intercourse as a risk factor for subsequent HPV infection in adolescent and young adult women. *J Pediatr, 141*(5), 718-723. doi:10.1067/mpd.2002.128893
52. Kapeu, A. S., Luostarinen, T., Jellum, E., Dillner, J., Hakama, M., Koskela, P…..Lehtinen, M. (2009). Is Smoking an Independent Risk Factor for Invasive Cervical Cancer? A Nested Case-Control Study Within Nordic Biobanks. *Am J Epidemiol, 169*(4), 480-488. doi:10.1093/aje/kwn354

53. Kazerooni, T., & Mosalaee, A. (2002). Does contraceptive method change the Pap smear finding? *Contraception, 66*(4), 243-246. doi:10.1016/S0010-7824(02)00365-7
54. Kjellberg, L., Hallmans, G., Ahren, A., Johansson, R., Bergman, F., Wadell, G…Dillner, J. (2000). Smoking, diet, pregnancy and oral contraceptive use as risk factors for cervical intra-epithelial neoplasia in relation to human papillomavirus infection. *Br J Cancer, 82*(7), 1332-1338. doi:10.1054/bjoc.1999.1100
55. Koshiol, J., Lindsay, L., Pimenta, J. M., Poole, C., Jenkins, D., & Smith, J. S. (2008). Persistent Human Papillomavirus Infection and Cervical Neoplasia: A Systematic Review and Meta-Analysis. *Am J Epidemiol, 168*(2), 123-137. doi:10.1093/aje/kwn036
56. Koskela, P., Anttila, T., Bjørge, T., Brunsvig, A., Dillner, J., Hakama, M….Paavonen, J. (2000). Chlamydia trachomatis infection as a risk factor for invasive cervical cancer. *Int J Cancer, 85*(1), 35-39. doi:10.1002/(SICI)1097-0215(20000101)85:13.0.CO;2-A
57. Krüger-Kjær, S., van den Brule, Adriaan J.,C., Svare, E. I., Engholm, G., Sherman, M. E., Poll, P. A….Meijer, Chris J. L. M. (1998). Different risk factor patterns for high-grade and low-grade intraepithelial lesions on the cervix among HPV-positive and HPV-negative young women. *Int J Cancer, 76*(5), 613-619. doi:10.1002/(SICI)1097-0215(19980529)76:53.0.CO;2-T
58. La Vecchia, C., & Boccia, S. (2014). Oral contraceptives, human papillomavirus and cervical cancer. *Eur J Cancer Prev, 23*(2), 110-112. doi:10.1097/CEJ.0000000000000000
59. Lacey, J. V., Frisch, M., Brinton, L. A., Abbas, F. M., Barnes, W. A., Gravitt, P. E…..Hildesheim, A. (2001). Associations between Smoking and Adenocarcinomas and Squamous Cell Carcinomas of the Uterine Cervix (United States). *Cancer Causes Control, 12*(2), 153-161. doi:10.1023/A:1008918310055
60. Lehtinen, M., Apter, D., Baussano, I., Eriksson, T., Natunen, K., Paavonen, J…Dubin, G. (2014). Characteristics of a cluster-randomized phase IV human papillomavirus vaccination effectiveness trial. *Vaccine, 33*(10), 1284-1290. doi:10.1016/j.vaccine.2014.12.019
61. Lehtinen, M., Ault, K. A., Lyytikainen, E., Dillner, J., Garland, S. M., Ferris, D. G….Paavonen, J. (2011). Chlamydia trachomatis infection and risk of cervical intraepithelial neoplasia. *Sex Transm Infect, 87*(5), 372-376. doi:10.1136/sti.2010.044354
62. Lehtinen, M., Dillner, J., Knekt, P., Luostarinen, T., Aromaa, A., Kirnbauer, R….Hakama, M. (1996). Serologically diagnosed infection with human papillomavirus type 16 and risk for subsequent development of cervical carcinoma: nested case-control study. *BMJ, 312*(7030), 537-326. 1 doi:0.1136/bmj.312.7030.537
63. Lehtinen, M., Söderlund-Strand, A., Vänskä, S., Luostarinen, T., Eriksson, T., Natunen, K. . . . Garnett, G. (2018). Impact of gender-neutral or girls-only vaccination against human papillomavirus—Results of a community-randomized clinical trial (I). *Int J Cancer, 142*(5), 949-958. doi:10.1002/ijc.31119
64. Lehtinen, M., Surcel, H., Natunen, K., Pukkala, E., & Dillner, J. (2017). Cancer Registry follow-up for 17 million person-years of a nationwide maternity cohort. *Cancer Med, 6*(12), 3060-3064. doi:10.1002/cam4.1222

65. Lehtinen, M., Paavonen, J., Wheeler, C. M., Jaisamrarn, U., Garland, S. M., Castellsagué, X.. . . Dubin, G. (2012). Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. *Lancet Oncol, 13*(1), 89-99. doi:10.1016/S1470-2045(11)70286-8
66. Longatto-Filho, A., Hammes, L. S., Sarian, L. O., Roteli-Martins, C., Derchain, S. F. M., Eržen, M….Syrjänen, K. (2011). Hormonal Contraceptives and the Length of Their Use Are Not Independent Risk Factors for High-Risk HPV Infections or High-Grade CIN. *Gynecol Obstet Invest, 71*(2), 93-103. doi:10.1159/000320742
67. Louvanto, K., Eriksson, T., Gray, P., Apter, D., Baussano, I., Bly, A….Lehtinen, M. (2020). Baseline findings and safety of infrequent vs. frequent screening of human papillomavirus vaccinated women. *Int J Cancer, 147*(2), 440-447. doi:10.1002/ijc.32802
68. Luostarinen, T., Lehtinen, M., Bjørge, T., Abeler, V., Hakama, M., Hallmans, G….Hakulinen, T. (2004). Joint effects of different human papillomaviruses and Chlamydia trachomatis infections on risk of squamous cell carcinoma of the cervix uteri. *Eur J Cancer, 40*(7), 1058-1065. doi:10.1016/j.ejca.2003.11.032
69. Luostarinen, T., af Geijersstam, V., Bjørge, T., Eklund, C., Hakama, M., Hakulinen, T….Lehtinen, M. (1999). No excess risk of cervical carcinoma among women seropositive for both HPV16 and HPV6/11. *Int J Cancer, 80*(6), 818-822. doi:10.1002/(SICI)1097-0215(19990315)80:63.0.CO;2-T
70. Luostarinen, T., Namujju, P. B., Merikukka, M., Dillner, J., Hakulinen, T., Koskela, P….Lehtinen, M. (2013). Order of HPV/Chlamydia infections and cervical high-grade precancer risk: A case-cohort study. *Int J Cancer, 133*(7), 1756-1759. doi:10.1002/ijc.28173
71. Madeleine, M. M., Anttila, T., Schwartz, S. M., Saikku, P., Leinonen, M., Carter, J. J….Daling, J. R. (2007). Risk of cervical cancer associated with Chlamydia trachomatis antibodies by histology, HPV type and HPV cofactors. *Int J Cancer, 120*(3), 650-655. doi:10.1002/ijc.22325
72. Marks, M., Gravitt, P. E., Gupta, S. B., Liaw, K., Kim, E., Tadesse, A….Celentano, D. D. (2011). The association of hormonal contraceptive use and HPV prevalence. *Int J Cancer, 128*(12), 2962-2970. doi:10.1002/ijc.25628
73. Moreno, V., Bosch, F. X., Muñoz, N., Meijer, Chris J. L. M., Shah, K. V., Walboomers, J. M. M…..Franceschi, S. (2002). Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. *Lancet, 359*(9312), 1085-1092. doi:10.1016/S0140-6736(02)08150-3
74. National Cancer Institute. (n.d.). Retrieved from https://www.cancer.gov/publications/dictionaries/cancer-terms/def/cervical-intraepithelial-neoplasia-grade-2-3 [Accessed 15th December 2021]
75. Nielsen, A., Kjaer, S. K., Munk, C., Osler, M., & Iftner, T. (2010). Persistence of high-risk human papillomavirus infection in a population-based cohort of Danish women. *J.Med.Virol, 82*(4), 616-623. doi:10.1002/jmv.21750
76. NORDCAN. (2019). *Cancer statistics for Nordic countries*. Retrieved from https://www-dep.iarc.fr/NORDCAN/English/frame.asp

77. Oh, H. Y., Kim, M. K., Seo, S., & Lee, J. (2016). Association of Combined Tobacco Smoking and Oral Contraceptive Use With Cervical Intraepithelial Neoplasia 2 or 3 in Korean Women. *J Epidemiol, 26*(1), 22-29. doi:10.2188/jea.JE20150047
78. Paavonen, J. (2001). Chlamydia trachomatis and cancer. *Sex Transm Infect, 77*(3), 154-156. doi:10.1136/sti.77.3.154
79. Paavonen, J., Prof, Jenkins, D., Prof, Bosch, F. X., Naud, P., M.D., Salmerón, J., DrSC, Wheeler, C. M.. . . Dubin, G., M.D. (2007). Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. *Lancet, 369*(9580), 2161-2170. doi:10.1016/S0140-6736(07)60946-5
80. Peng, Y., Wang, X., Feng, H., & Yan, G. (2017). Is oral contraceptive use associated with an increased risk of cervical cancer? An evidence-based meta-analysis. *J Obstet Gynaecol Res, 43*(5), 913-922. doi:10.1111/jog.13291
81. Plummer, M., Herrero, R., Franceschi, S., Meijer, C. J. L. M., Snijders, P. J. F., Bosch, F. X.…Munoz, N. (2003). Smoking and Cervical Cancer: Pooled Analysis of the IARC Multi-Centric Case-Control Study. *Cancer Causes Control, 14*(9), 805-814. doi:10.1023/B:CACO.0000003811.98261.3e
82. Rimpelä, A.,H., Rimpelä, M.,K., & Kosunen, E. A. (1992). Use of oral contraceptives by adolescents and its consequences in Finland 1981-91. *BMJ, 305*(6861), 1053-1057. doi:10.1136/bmj.305.6861.1053
83. Roura, E., Castellsagué, X., Pawlita, M., Travier, N., Waterboer, T., Margall, N.. . . Riboli, E. (2014). Smoking as a major risk factor for cervical cancer and pre-cancer: Results from the EPIC cohort. *Int J Cancer, 135*(2), 453-466. doi:10.1002/ijc.28666
84. Roura, E., Travier, N., Waterboer, T., de Sanjosé, S., Bosch, F. X., Pawlita, M... . . Castellsagué, X. (2016). The Influence of Hormonal Factors on the Risk of Developing Cervical Cancer and Pre-Cancer : Results from the EPIC Cohort. *PLoS One, 11*(1), e0147029. doi:10.1371/journal.pone.0147029
85. Rousseau, M., Franco, E. L., Villa, L. L., Sobrinho, J. P., Termini, L., Prado, J. M., & Rohan, T. E. (2000). A Cumulative Case-Control Study of Risk Factor Profiles for Oncogenic and Nononcogenic Cervical Human Papillomavirus Infections. *Cancer Epidemiol Biomarkers Prev, 9*(5), 469-476.
86. Ruiz, Á M., Ruiz, J. E., Gavilanes, A. V., Eriksson, T., Lehtinen, M., Pérez, G…..Haupt, R. M. (2012). Proximity of First Sexual Intercourse to Menarche and Risk of High-Grade Cervical Disease. *J Infect Dis, 206*(12), 1887-1896. doi:10.1093/infdis/jis612
87. Safaeian, M., Quint, K., Schiffman, M., Rodriguez, A. C., Wacholder, S., Herrero, R.…Burk, R. D. (2010). Chlamydia trachomatis and Risk of Prevalent and Incident Cervical Premalignancy in a Population-Based Cohort. *JNCI, 102*(23), 1794-1804. doi:10.1093/jnci/djq436
88. Salazar, E. L., González, J. L., Olmos, A., & Calzada, L. (2005). Influence of the use of oral contraceptives as risk factors for human papillomavirus infection and cervical intraepithelial neoplasia. *Ginecol Obstet Mex, 73*(2), 83.
89. Samoff, E., Koumans, E. H., Markowitz, L. E., Sternberg, M., Sawyer, M. K., Swan, D….Unger, E. R. (2005). Association of Chlamydia trachomatis with Persistence of High-Risk Types of Human Papillomavirus in a Cohort of Female Adolescents. *Am J Epidemiol, 162*(7), 668-675. doi:10.1093/aje/kwi262

90. Schiffman, M. H., Bauer, H. M., Stanton, C. K., Manos, M. M., Hoover, R. N., Glass, A. G….Wacholder, S. (1993). Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. *JNCI, 85*(12), 958-964. doi:10.1093/jnci/85.12.958
91. Schiffman, M., & Wentzensen, N. (2013). Human papillomavirus (HPV) infection and the multi-stage carcinogenesis of cervical cancer. *Cancer Epidemiol Biomarkers Prev, 22*(4), 553-560. doi:10.1158/1055-9965.EPI-12-1406
92. Seraceni, S., De Seta, F., Colli, C., Del Savio, R., Pesel, G., Zanin, V….Comar, M. (2014). High prevalence of hpv multiple genotypes in women with persistent chlamydia trachomatis infection. *Infect Agent Cancer, 9*(1), 30. doi:10.1186/1750-9378-9-30
93. Shapiro, S., Rosenberg, L., Hoffman, M., Kelly, J. P., Cooper, D. D., Carrara, H…..Williamson, A. (2003). Risk of Invasive Cancer of the Cervix in Relation to the Use of Injectable Progestogen Contraceptives and Combined Estrogen/Progestogen Oral Contraceptives (South Africa). *Cancer Causes Control, 14*(5), 485-495. doi:10.1023/A:1024910808307
94. Shew, M. L., Fortenberry, J. D., Miles, P., & Amortegui, A. J. (1994). Interval between menarche and first sexual intercourse, related to risk of human papillomavirus infection. *J Pediatr, 125*(4), 661-666. doi:10.1016/S0022-3476(94)70031-1
95. Silva, J., Cerqueira, F., & Medeiros, R. (2013). Chlamydia trachomatis infection: implications for HPV status and cervical cancer. *Arch Gynecol Obstet, 289*(4), 715-723. doi:10.1007/s00404-013-3122-3
96. Smith, J. S., Bosetti, C., Munoz, N., Herrero, R., Bosch, F. X., Eluf-Neto, J….Peeling, R. W. (2004). Chlamydia trachomatis and invasive cervical cancer: a pooled analysis of the IARC multicentric case-control study. *Int J Cancer, 111*(3), 431-9. doi:10.1002/ijc.20257
97. Smith, J. S., Green, J., de Gonzalez, A. B., Appleby, P., Peto, J., Plummer, M….Beral, V. (2003). Cervical cancer and use of hormonal contraceptives: a systematic review. *Lancet, 361*(9364), 1159-1167. doi:10.1016/S0140-6736(03)12949-2
98. Smith, J. S., Muñoz, N., Herrero, R., Eluf-Neto, J., Ngelangel, C., Franceschi, S….Peeling, R. W. (2002). Evidence for Chlamydia trachomatis as a Human Papillomavirus Cofactor in the Etiology of Invasive Cervical Cancer in Brazil and the Philippines. *J Infect Dis, 185*(3), 324-331. doi:10.1086/338569
99. Solomon, D., Davey, D., Kurman, R., Moriarty, A., O'Connor, D., Prey, M….. for the Forum Group Members and the Bethesda 2001 Workshop. (2002). The 2001 Bethesda System: Terminology for Reporting Results of Cervical Cytology. *JAMA, 287*(16), 2114-2119. doi:10.1001/jama.287.16.2114
100. Steben, M., & Duarte-Franco, E. (2007). Human papillomavirus infection: Epidemiology and pathophysiology. *Gynecol Oncol, 107*(2), S2-S5. doi:10.1016/j.ygyno.2007.07.067
101. Syrjänen, K., Yliskoski, M., Kataja, V., Hippeläinen, M., Syrjänen, S., Saarikoski, S., & Ryhänen, A. (1990). Prevalence of Genital Human Papillomavirus Infections in a Mass-Screened Finnish Female Population Aged 20–65 Years. *International Journal of STD & AIDS*, *1*(6), 410–415.

102. Szarewski, A., Sasieni, P., Edwards, R., Cuzick, J., Jarvis, M. J., Steele, S. J…..Guillebaud, J. (1996). Effect of smoking cessation on cervical lesion size. *Lancet, 347*(9006), 941-943. doi:10.1016/S0140-6736(96)91417-8
103. Trimble, C. L., Genkinger, J. M., Burke, A. E., Hoffman, S. C., Helzlsouer, K. J., Diener-West, M….Alberg, A. J. (2005). Active and Passive Cigarette Smoking and the Risk of Cervical Neoplasia. *Obstet Gynecol, 105*(1), 174-181. doi:10.1097/01.AOG.0000148268.43584.03
104. Vaccarella, S., Herrero, R., Dai, M., Snijders, P. J. F., Meijer, Chris J. L. M., Thomas, J. O….Franceschi, S. (2006). Reproductive Factors, Oral Contraceptive Use, and Human Papillomavirus Infection: Pooled Analysis of the IARC HPV Prevalence Surveys. *Cancer Epidemiol Biomarkers Prev, 15*(11), 2148-2153. doi:10.1158/1055-9965.EPI-06-0556
105. Walboomers, J. M. M., Jacobs, M. V., Manos, M. M., Bosch, F. X., Kummer, J. A., Shah, K. V…..Muñoz, N. (1999). Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J.Pathol, 189*(1), 12-19. doi:10.1002/(SICI)1096-9896(199909)189:13.0.CO;2-F
106. Wallin, K., Wiklund, F., Luostarinen, T., Ångström, T., Anttila, T., Bergman, F….Dillner, J. (2002). A population-based prospective study of Chlamydia trachomatis infection and cervical carcinoma. *Int J Cancer, 101*(4), 371-374. doi:10.1002/ijc.10639
107. Wallin, K., Wiklund, F., Ångström, T., Bergman, F., Stendahl, U., Wadell, G….Dillner, J. (1999). Type-Specific Persistence of Human Papillomavirus DNA before the Development of Invasive Cervical Cancer. *N Engl J Med, 341*(22), 1633-1638. doi:10.1056/NEJM199911253412201
108. Winkelstein, J., W. (1977). Smoking and cancer of the uterine cervix: hypothesis. *Am J Epidemiol, 106*(4), 257-259. doi:10.1093/oxfordjournals.aje.a112460
109. World Health Organization (2010. A healthy lifestyle-WHO recommendations. Retrieved from https://www.who.int/europe/news-room/fact-sheets/item/a-healthy-lifestyle---who-recommendations / [Accessed 15th August 2022]
110. World Health Organization. (2020). Sexually Transmitted Infections (STIs). Retrieved from https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections-(stis) [Accessed 15th May 2022]
111. Xu, H., Egger, S., Velentzis, L. S., O'Connell, D.,L., Banks, E., Darlington-Brown, J….Sitas, F. (2018). Hormonal contraceptive use and smoking as risk factors for high-grade cervical intraepithelial neoplasia in unvaccinated women aged 30–44 years: A case-control study in New South Wales, Australia. *Cancer Epidemiol, 55*, 162-169. doi:10.1016/j.canep.2018.05.013
112. Ylitalo, N., Sørensen, P., Josefsson, A. M., Magnusson, P. K. E., Andersen, P. K., Pontén, J….Melbye, M. (2000). Consistent high viral load of human papillomavirus 16 and risk of cervical carcinoma in situ: a nested case-control study. *Lancet, 355*(9222), 2194-2198. doi:10.1016/S0140-6736(00)02402-8
113. zur Hausen, H., Meinhof, W., Scheiber, W., & Bornkamm, G. W. (1974). Attempts to detect virus-secific DNA in human tumors. I. Nucleic acid hybridizations with complementary RNA of human wart virus. *Int J Cancer, 13*(5), 650-656.

PUBLICATIONS

PUBLICATION I

Prolonged oral contraceptive use and risk of acquisition of human papillomavirus type 16/18 infections

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The risk of cervical atypia in oral contraceptive users.

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PUBLICATION III

Is the risk of cervical atypia associated with the interval between menarche and the start of sexual activity? A population-based cohort study

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Is the risk of cervical atypia associated with the interval between menarche and the start of sexual activity? A population-based cohort study

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ABSTRACT

Objective We investigated whether the risk of cervical atypia is associated with a short interval between the age at first sexual intercourse (FSI) or age at the start of oral contraceptive (OC) use and menarche.

Design A population-based cohort study.

Setting Finnish women in the age range of 16–17 years old were enrolled in the PATRICIA trial of human papillomavirus (HPV) 16/18 vaccine efficacy.

Participants The association of cervical atypia with the interval between FSI or start of OC use and menarche was assessed in the control arm (hepatitis A vaccinated) who had participated in biannual clinical follow-up visits for 4 years. Altogether, 913 women had normal baseline cervical cytology and answered behavioural questionnaires at enrolment and end of the follow-up.

Main outcome measure ORs with 95% CIs using univariate and multivariable logistic regression were used to assess the association between cervical atypia and the interval between FSI or the start of OC use and menarche.

Results The mean ages at menarche, FSI and the start of OC use were 12.4, 16.0 and 16.4. *Chlamydia trachomatis* infection was associated with an increased risk of cervical atypia in women with a short (<3 years) interval between menarche and FSI/start of OC use (OR 1.8, 95% CI 1.0 to 3.6 and OR 2.2, 95% CI 1.0 to 5.1). Whereas HPV 16/18 infection was associated with increased atypia risk estimates in women with a longer (≥3 years) interval (OR 1.8, 95% CI 1.1 to 2.7 and OR 1.4, 95% CI 1.0 to 2.1). In women with a short interval between menarche and FSI, early age at the start of OC use was not associated with an increased risk of cervical atypia in the univariate (OR 0.7) nor multivariable analyses.

Conclusion Short interval between menarche and the age at start of sexual activity does not increase the risk of HPV-associated cervical atypia.

Trial registration number NCT00122681.

Strengths and limitations of this study

- ► A large human papillomavirusHPV vaccination trial cohort with standardised clinical and laboratory procedures.
- ► The repeated self-reported study questionnaires were comprehensive and less subject to recall bias.
- ► Use of the overall cervical atypia endpoint increases study power but may have diluted the effects.

INTRODUCTION

Sexually transmitted human papillomavirus (HPV) infections cause both cytological and histological cervical abnormalities.[1 2] Clinical manifestations of persistent infection with oncogenic HPV types and squamous intraepithelial lesions (SILs) of the cervix, also known as cervical intraepithelial neoplasia (CIN), are the precursors of invasive cervical cancer (ICC).[3–5] In addition to HPV, other risk factors which may play a role in the pathogenesis of ICC are smoking,[6] *Chlamydia trachomatis*,[7] lifetime number of sexual partners (LNSPs),[8] age at first sexual intercourse (FSI),[9] parity[10] and the use of oral contraceptives (OCs).[11]

Both early age at FSI and early age at the start of OC use are associated with an increased risk of SIL and CIN.[9 12–14] Furthermore, a short lag between menarche and FSI is a risk factor of SIL/CIN.[9 12 14] This is probably due to exposure of immature cervical cells to infection with HPV, as persistent infections with oncogenic HPV types are established more readily in an immature cervix.[13 15] However, whether or not early start of OC use has an independent role here is unknown. The interplay of the time interval between age at the start of OC use or FSI and menarche in cervical carcinogenesis has not been studied.

In a large cohort study, we have investigated whether the risk of cervical atypia is associated with a short interval between menarche and the age at the start of OC use or FSI.

Table 1 Characteristics of 22-year-old women (n=913) who attended eight biannual follow-up visits and a subgroup of these women (n=197) who developed cervical atypia during 4 years of follow-up

	Attendees		Women with atypia	
Characteristics	**n=913**	**%**	**n=197**	**%**
Age				
22	422	46.2	94	47.7
23	489	53.6	103	52.3
24	2	0.2	0	0
Missing	0	0	0	0
Age at menarche				
≤11	194	21.3	52	26.4
12–14	659	72.2	136	69.0
≥15	52	5.7	6	3.10
Missing	8	0.8	3	1.5
Age at FSI				
12–16	602	65.9	129	65.5
17–22	273	29.9	60	30.5
Missing	38	4.2	8	4.0
LNSPs				
0	3	0.3	1	0.5
1	131	14.3	29	14.7
2–4	283	31.0	57	28.9
5–9	236	25.9	50	25.4
≥10	230	25.2	55	28.0
Missing	30	3.3	5	2.5
OC use				
Non-user	62	6.8	15	7.6
User	842	92.2	179	90.9
Missing	9	1.0	3	1.5
Age at start of OC use				
12–16	504	55.2	104	52.8
17–22	371	40.6	85	43.1
Missing	38	4.2	8	4.1
Condom use				
Non-user	414	45.4	97	49.2
User	406	44.5	83	42.1
Don't know	76	8.3	16	8.1
Missing	17	1.8	1	0.5
Smoking				
Never	525	57.5	108	54.8
Past	93	10.2	16	8.1
Present	291	31.9	73	37.1
Missing	4	0.4	0	0
HPV 16				
Negative	711	77.9	145	73.6
Positive	201	22.0	52	26.4
HPV 18				
Negative	792	86.8	165	83.8
Positive	120	13.1	32	16.2
Chlamydia				
Negative	811	88.8	175	88.8
Positive	102	11.2	22	11.2

FSI, first sexual intercourse; HPV, human papillomavirus; LNSPs, lifetime number of sexual partners; OC, oral contraceptive.

MATERIALS AND METHODS

Study sample

The study population consists of women enrolled in the control arm of a double-blinded, multi-national randomised control PATRICIA trial whose primary aim was to evaluate the vaccine efficacy of the HPV 16/18 vaccine against CIN2+.[16 17] Full description of the trial, details of recruitment and final results on its endpoints have been reported earlier.[18] PATRICIA enrolled only 16–17-year-old women in Finland (2409 received at least one dose of HPV 16/18 vaccine) and 2399 women (received at least one dose of hepatitis A virus (HAV) vaccine). The criteria of having no more than six LNSPs was not applied in Finland, so all women interested and willing to participate in the study were included.[18] Written informed consent was obtained from all the participants.

The present study began after the end of the clinical PATRICIA trial. All 4808 women who were approximately 22 years old when exiting the trial were sent a questionnaire on living conditions, lifestyle habits and sexual health. All the women (913) who had received the HAV vaccine, answered the questionnaires both at enrolment and at the end of the follow-up, and had negative cytology at baseline and before menarche were eligible (table 1). Cytology outcomes were detected at the follow-up visits.

Data collection

In addition to collecting information on living conditions and lifehabits, the questionnaires collected information about history of OC use, use of other contraceptives, smoking, menarche and sexual habits. The end of study questionnaire was more complete regarding the initiation of sexual habits, and was therefore used in the analysis. The age at the start of OC use, menarche and age at FSI were the independent variables in this study. Intervals of <3 years, or more than or equal to 3 years were calculated between menarche and the age at the start of OC use, as well as between menarche and FSI. Data on smoking ('never smokers', 'past smokers' and 'present smokers'), LNSPs ('none', '1', '2–4', '5–9' and 'more than 10'), condom use ('non-user', 'user' and 'do not know') and sexually transmitted infections (HPV

Table 2 Distribution of cervical atypia risk factors by interval between menarche and age at the start of OC use or age at the FSI in young adult women followed up for 4 years

	Interval between menarche and age at the start of OC use		Interval between menarche and the FSI	
	Interval <3 years	Interval ≥3 years	Interval <3 years	Interval ≥3 years
	(n=192)	(n=675)	(n=302)	(n=566)
Category	n/Mean (%/SD)	n/Mean (%/SD)	n/Mean (%/SD)	n/Mean (%/SD)
Chlamydia trachomatis	39 (20.3)	59 (8.7)	54 (18.0)	44 (7.8)
HPV 16	55 (28.7)	142 (21.1)	97 (32.2)	100 (17.8)
HPV 18	35 (18.2)	83 (12.3)	47 (15.6)	71 (12.5)
HPV 16/18	69 (35.9)	190 (28.2)	115 (38.2)	144 (25.4)
Smoking				
Never	84 (43.8)	405 (60.4)	134 (44.8)	356 (63.0)
Past smoker	22 (11.4)	69 (10.2)	37 (12.4)	54 (9.6)
Present smoker	86 (44.8)	197 (29.4)	128 (42.8)	155 (27.4)
Age at menarche	13.4 (1.2)	12.2 (1.1)	13.1 (1.3)	12.1 (1.1)
Age at FSI	14.7 (1.2)	16.3 (1.9)	14.6 (1.2)	16.7 (1.9)
Age at start of OC use	14.9 (1.2)	16.9 (1.7)	15.3 (1.3)	17.1 (1.7)
Lifetime number of partners				
0	0	1 (0.2)	0	1 (0.2)
1	11 (5.7)	119 (17.6)	14 (4.6)	116 (20.5)
2–4	46 (24.0)	230 (34.1)	73 (24.2)	204 (36.0)
5–9	69 (36.0)	163 (24.2)	98 (32.5)	134 (23.7)
>10	66 (34.4)	162 (24.0)	117 (38.7)	111 (19.6)

FSI, first sexual intercourse; HPV, human papillomavirus; OC, oral contraceptive.

16/18 and *C. trachomatis*) were used as covariables, as they are important factors in cervical carcinogenesis. These covariables were used in both the univariate and multivariable models to evaluate if the short intervals between menarche and FSI or age at the start of OC use are truly associated with or modify the risk of cervical atypia.

Laboratory analysis and endpoints

In the PATRICIA trial, biannual cervical cytological and DNA samples were obtained in conjunction with pelvic examination. PCR analyses for *C. trachomatis* and HPV DNA were performed as described.[18]

At the follow-up visits, the first cytological findings of atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL) were registered as index incident cases for the statistical analysis. Colposcopy-directed biopsy samples were also obtained during the trial. The first histopathological findings of CIN grades 1, 2 and 3 were also listed as the index cases for statistical analysis. SIL and CIN cases were combined together to form a new variable, cervical atypia.

Cervical atypia findings were registered by the interval between menarche and FSI or the start of OC use to form four mutually exclusive different individual outcome variables; (1) cervical atypia with shorter than 3 years lag between menarche and FSI, (2) cervical atypia with equal or longer than 3 years lag between menarche and FSI, (3) cervical atypia with shorter than 3 years lag between menarche and OC use and (4) cervical atypia with equal or longer than 3 years lag between menarche and OC use.

Patient and public involvement

Patient (adolescent study subjects) and public (parental) involvement in the planning and design of the study was noted as their attitudes and willingness to participate in a HPV vaccination trial in a questionnaire sent to households (parents and their adolescent daughter) in one of the major study site communities.[19] No patients with cervical cytological atypia were involved in setting the research questions, the outcome measures or in developing the plans for recruitment, design or implementation of the study.

There are no plans to directly disseminate the results of the research to study participants; however, the results have and will be disseminated to a wider audience, including members of the public, patients, health professionals and experts through written communication, events and conferences, networks and social media.

Table 3 Relative risk (ORs with 95% CI) of cervical atypia (cytological SIL and/or CIN grade 1 or worse, CIN1+) associated with different covariables in analyses stratified by the interval between menarche and age at FSI (category 1), or between menarche and the age at start of OC (category 2) use in young adult women followed up for 4 years

	Frequency and relative risk of cytological atypia: SIL or CIN1 in the different categories by interval from menarche							
	Category 1				Category 2			
	Menarche to FSI <3 years SIL/CIN1+		Menarche to FSI ≥3 years SIL/CIN1+		Menarche to start of OCs <3 years SIL/CIN1+		Menarche to start of OCs ≥3 years SIL/CIN1+	
Variable	n/N	OR (95% CI)	n/N	OR (95% CI)	n/N	OR (95% CI)	n/N	OR (95% CI)
HPV 16/18								
Positive	21/115	1.0 (0.6 to 2.0)	45/144	1.8 (1.1 to 2.7)	13/69	1.4 (0.6 to 3.0)	53/190	1.4 (1.0 to 2.1)
Negative	33/186	1	87/422	1	18/123	1	102/484	1
Chlamydia								
Positive	14/54	1.8 (1.0 to 3.6)	8/44	0.7 (0.3 to 1.6)	10/39	2.2 (1.0 to 5.1)	12/59	0.8 (0.4 to 1.6)
Negative	40/248	1	124/522	1	21/153	1	143/616	1
Smoking								
Yes	34/165	1.5 (0.8 to 2.7)	52/209	1.1 (0.8 to 1.7)	22/108	2.1 (1.0 to 5.0)	64/266	1.1 (0.8 to 1.6)
No	20/134	1	80/356	1	9/84	1	91/405	1
Condom use								
Yes	22/146	0.7 (0.4 to 1.2)	59/256	0.9 (0.6 to 1.3)	15/102	0.6 (0.3 to 1.3)	66/300	0.9 (0.6 to 1.3)
No	29/139	1	61/251	1	16/80	1	74/309	1
LNSPs								
High	39/215	1.1 (0.6 to 2.0)	64/245	1.3 (0.9 to 2.0)	24/135	1.5 (0.6 to 3.8)	79/325	1.2 (0.8 to 1.7)
Low	15/87	1	68/321	1	7/57	1	76/350	1

*Five or more.
CIN, cervical intraepithelial neoplasia; FSI, first sexual intercourse; LNSPs, lifetime number of sexual partners; OC, oral contraceptive; SIL, squamous intraepithelial lesion.

Table 4 Relative risk (ORs with 95% CI) of cervical atypia (cytological SIL and/or CIN grade 1 or worse, CIN1+) stratified by the interval between menarche and age at FSI or between menarche and the age at start of OC use in young adult women followed up for 4 years

Frequency and relative risk of cytological atypia: SIL or CIN1 in the different categories by interval from menarche						
	Category 1			Category 2		
	Menarche to FSI <3 years SIL/CIN1+			Menarche to start of OCs ≥3 years SIL/CIN1+		
Variable	n/N	OR (95% CI)		n/N	OR (95% CI)	
Lag between FSI and menarche						
<3 years.	53/301	NA		23/110	0.9 (0.5 to 1.4)	0.9 (0.8 to 1.0)
≥3 years	NA	NA		132/565	1	*Interval (cont.)
Lag between start of OCs and menarche						
<3 years.	30/191	0.7 (0.4 to 1.3)	0.9 (0.9 to 1.0)	NA	NA	
≥3 years	23/110	1	*Interval (cont.)	155/675	NA	

CIN, cervical intraepithelial neoplasia; FSI, first sexual intercourse; OC, oral contraceptive; SIL, squamous intraepithelial lesion.

Statistical analysis

The outcome variables were analysed in the univariate and multivariable logistic regression models along with the independent variables and above listed covariates. The risks are reported as the ORs with 95% CI. The statistical analysis was performed using Stata V.14.0 (Stata Corp LP, Statistical Software: Release 14).

RESULTS

Baseline characteristics of our study cohort attending biannual follow-up visits for 4 years are materially homogeneous with little variation (table 1).

Age at menarche was between 12 and 14 years for 659 (72.2%) participants. Age at the FSI and age at the start of OC use were between 12 and 16 years for 602 (65.9%) and 504 (55.2%) participants, respectively. No cervical atypia cases were found before the menarche, age at the FSI or the age at the start of OC use. One cervical atypia case occurring concomitantly with the start of OC use was removed from the analyses.

By the end of the follow-up period, out of 913 women, 156 (17.1%) had ASCUS, 189 (20.7%) had LSIL, 5 (0.6%) had HSIL, 40 (4.4%) had CIN1, 22 (2.41%) had CIN2 and 8 (0.9%) had CIN3. 197 (21.6%) of 913 women were identified with cervical atypia (table 1). Almost one-third of the women with cervical atypia (55 (28.0%) of 197) had more than 10 LNSPs. Half of the women with or without cervical atypia, 49.2% and 45.4% respectively, did not regularly use condoms. Most of the women (179 (90.9%) of 197) with cervical atypia had used OCs. Age at the start of OC use for the majority of these women (104 (52.8%) of 197) was between 12 and 16 years (table 1). During the 4 year follow-up, 201 (22%) of all women were tested positive for HPV 16 and 120 (13.1%) were tested positive for HPV 18 (table 1). One-third of women with either HPV 16 or HPV 18, or both were diagnosed with cervical atypia during the follow-up. The number of women who tested positive for *C. trachomatis* was 102 (11.2%), and the number of *C. trachomatis* positive women with cervical atypia was 22 (11.2%) (table 1).

We categorised the risk factors of cervical atypia according to the interval between menarche and age at the start of OC use, or between menarche and age at FSI using a stratification of <3 years and ≥3 years (table 2).

The mean ages at menarche, at FSI and at the start of OC use were similar in the corresponding categories (table 2). Women in the <3 years interval categories were more often HPV 16 positive than women in the ≥3 years interval categories (table 2). The percentages of women with multiple (>5) LNSPs were also higher in the short interval categories (table 2).

In the univariate analysis, the risk of cervical atypia associated with its known risk factors was evaluated separately in the short and long interval categories (table 3).

Cervical atypia risk estimates associated with HPV 16/18 were increased (OR 1.8, 95% CI 1.1 to 2.7 and OR 1.4, 95% CI 1.0 to 2.1) in the longer (≥3 years) interval categories. On the contrary, the cervical atypia risk associated with *C. trachomatis* was increased (OR 1.8, 95% CI 1.0 to 3.6 and OR 2.2, 95% CI 1.0 to 5.1) in the short (<3 years) interval categories. Condom use was not associated with a significantly decreased risk of cervical atypia in any of the interval categories (table 3).

In univariate analyses, the risk of cervical atypia associated with the short interval between menarche and age at the start of OC use appeared to be somewhat decreased (OR 0.7, 95% CI 0.4 to 1.3) when the interval between menarche and age at the FSI was short (table 4).

The risk estimate, however, approached unity (OR 0.9) when the interval was estimated as a continuous variable. There was no risk of atypia associated with the long-term interval between menarche and the start of OC use (table 4).

In multivariable analyses, stepwise exclusion of one variable at a time from the multivariable model was performed to check the interdependency of the interval between menarche, age at the start of OC use and age at FSI in this context. Exclusion of any of the abovementioned variables did not affect significance of the estimates (data not shown).

DISCUSSION

We found that cervical atypia was not associated with early start of sexual activity after menarche. The risk of cervical atypia associated with *C. trachomatis* was increased shortly after start of sexual activity following menarche, whereas the risk of cervical atypia was associated with HPV 16/18 infections more than 3 years after the start of sexual activity following menarche.

Our large HPV-vaccination-trial-derived population of young adult women, with uniform ethnicity (97% Caucasian Finnish women), and the standardised clinical and laboratory procedures are noteworthy strengths of the study. In young Finnish women, HIV infection has been and is extremely rare (www.thl.fi). Furthermore, over the entire follow-up period the trial participants received regular sexual health counselling which probably helped in retaining the participants and reduced possible confounding and bias in our study. To the best of our knowledge, the association between interval between menarche and the age at the start of OC use with cervical atypia has now been assessed for the first time.

Some limitations of our study are as follows. The use of the overall cervical atypia endpoint, which was necessary to retain the statistical power of the study strata. The study questionnaires used were self-reported at the ages of 18 and 22 years, the latter of which is subject to recall bias. The endpoint questionnaire (at age 22) was, however, in line with the enrolment questionnaire (at age 18), for example, for menarche. Moreover, questionnaire-based information regarding sexual behaviour is supposed to have adequate validity and reliability.[20 21] It gave the most comprehensive information about sexual risk-taking characteristics of the study subjects over time. This was important when assessing the longitudinal effects of OC use on prospective development of cervical atypia following the exposures. Free contraceptives were distributed to the participants during the trial period, which might have increased the proportions of OC and condom users in our study.

The absence of HPV 16/18 associated risk of cervical atypia in women with short lag between menarche and the start of sexual activity appears to defy the assumption that the immature cervical transformation zone is especially prone to persistent HPV infection.[15] Our observation is in line with Collins *et al*, who reported that the increased interval between menarche and the age at the FSI increases the risk of HPV infection.[22] Overall cervical atypia, the most common clinical manifestation of genital HPV infection, needs some time to develop.

On the other hand, our findings seem to contradict a study by Ruiz *et al* who first reported that short interval between menarche and age at the FSI is a predictor of cervical cytological abnormalities and CIN.[9] While our homogeneous study population had ampler power to detect a threefold increased risk (see online supplementary appendix), their study population was heterogeneous and had only baseline sexual risk-taking behaviour questionnaire data, which could not elaborate (possible changes in) the risk-taking behaviour during the follow-up. Furthermore, we found a lack of association between short interval of menarche and two different measures of the start of sexual activity (age at FSI and age at the start of OC use). However, these different observations on the interval between menarche and start of sexual activity, and the risk of cervical atypia,[9 12–14] may also reflect limited sample sizes.

Our group has earlier reported that when *C. trachomatis* infection precedes or cooccurs with HPV infection the risk of high-grade cervical neoplasia associated with the joint infection is very high.[23] Our results on the increased risk of *C. trachomatis* infection with cervical atypia especially in women with a short lag between menarche and the start of sexual activity emphasise the need to identify, treat and follow-up adolescent females with *C. trachomatis*.

In conclusion, while our study does not support the hypothesis that a short interval between menarche and age at the start of sexual activity always increases the risk of cervical atypia, early age of acquiring *C. trachomatis* infections may set the stage for cervical carcinogenesis and should be identified and treated.

Contributors IA developed the research protocol, analysed the data and prepared the manuscript. TE contributed in data acquisition and data interpretation. TL contributed in the analysis plan, commented on the drafts of the paper and helped in the revision of paper. DA commented on the tables and drafts of the paper. ML helped in data acquisition, contributed in the development of research plan, analysis plan, commented on the draft of the paper and the revision of the paper.

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REFERENCES

1. Brinton LA, Fraumeni JF. Epidemiology of uterine cervical cancer. *J Chronic Dis* 1986;39:1051–65.
2. Campion MJ, McCance DJ, Cuzick J, *et al*. Progressive potential of mild cervical atypia:prospective cytological, colposcopic, and virological study. *Lancet* 1986;8501:237–40.

3. Koutsky LA, Holmes KK, Critchlow CW, *et al*. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. *N Engl J Med* 1992;327:1272–8.
4. GY H, Burk RD, Klein S, *et al*. Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. *J Natl Cancer Inst* 1995;81:1365–71.
5. Liaw KL, Glass AG, Manos MM, *et al*. Detection of human papillomavirus DNA in cytologically normal women and subsequent cervical squamous intraepithelial lesions. *J Natl Cancer Inst* 1999;91:954–60.
6. Kapeu AS, Luostarinen T, Jellum E, *et al*. Is smoking an independent risk factor for invasive cervical cancer? A nested case-control study within Nordic biobanks. *Am J Epidemiol* 2009;169:480–8.
7. Lehtinen M, Ault KA, Lyytikainen E, *et al*. *Chlamydia trachomatis* infection and risk of cervical intraepithelial neoplasia. *Sex Transm Infect* 2011;87:372–6.
8. International Collaboration of Epidemiological Studies of Cervical Cancer. Cervical carcinoma and sexual behavior: collaborative reanalysis of individual data on 15,461 women with cervical carcinoma and 29,164 women without cervical carcinoma from 21 epidemiological studies. *Cancer Epidemiol Biomarkers Prev* 2009;18:1060–9.
9. Ruiz Ángela María, Ruiz JE, Gavilanes AV, *et al*. Proximity of first sexual intercourse to menarche and risk of high-grade cervical disease. *J Infect Dis* 2012;206:1887–96.
10. International Collaboration of Epidemiological Studies of Cervical Cancer. Cervical carcinoma and reproductive factors: collaborative reanalysis of individual data on 16,563 women with cervical carcinoma and 33,542 women without cervical carcinoma from 25 epidemiological studies. *Int J Cancer* 2006;119:1108–24.
11. Appleby P, Beral V, Berrington de González A, *et al*. Cervical cancer and hormonal contraceptives: collaborative reanalysis of individual data for 16,573 women with cervical cancer and 35,509 women without cervical cancer from 24 epidemiological studies. *Lancet* 2007;370:1609–21.
12. Shew ML, Fortenberry JD, Miles P, *et al*. Interval between menarche and first sexual intercourse, related to risk of human papillomavirus infection. *J Pediatr* 1994;125:661–6.
13. Daling JR, Madeleine MM, McKnight B, *et al*. The relationship of human papillomavirus-related cervical tumors to cigarette smoking, oral contraceptive use, and prior herpes simplex virus type 2 infection. *Cancer Epidemiol Biomarkers Prev* 1996;7:541–8.
14. Kahn JA, Rosenthal SL, Succop PA, *et al*. The interval between menarche and age of first sexual intercourse as a risk factor for subsequent HPV infection in adolescent and young adult women. *J Pediatr* 2002;141:718–23.
15. Ho GY, Bierman R, Beardsley L, *et al*. Natural history of cervicovaginal papillomavirus infection in young women. *N Engl J Med* 1998;338:423–8.
16. Adhikari I, Eriksson T, Luostarinen T, *et al*. The risk of cervical atypia in oral contraceptive users. *Eur J Contracept Reprod Health Care* 2018;23:12–17.
17. LehtinenM, PaavonenJ W*et al*. Overall efficacy of HPV-16/18 vaccine against the most stringent cervical pre-cancer end-points: end-of study report of a double blind, randomized trial. *Lancet Oncol* 2012;13:89–99.
18. Paavonen J, Jenkins D, Bosch FX, *et al*. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. *Lancet* 2007;369:2161–70.
19. Woodhall SC, Lehtinen M, Verho T, *et al*. Anticipated acceptance of HPV vaccination at the baseline of implementation: a survey of parental and adolescent knowledge and attitudes in Finland. *J Adolesc Health* 2007;40:466–9.
20. Kahn JA, Goodman E, Kaplowitz RA, *et al*. Validity of adolescent and young adult self-report of Papanicolaou smear results. *Obstet Gynecol* 2000;96:625–31.
21. Brener ND, Collins JL, Kann L, *et al*. Reliability of the youth risk behavior survey questionnaire. *Am J Epidemiol* 1995;141:575–80.
22. Collins SI, Mazloomzadeh S, Winter H, *et al*. Proximity of first intercourse to menarche and the risk of human papillomavirus infection: a longitudinal study. *Int J Cancer* 2005;114:498–500.
23. Luostarinen T, Lehtinen M, Bjørge T, *et al*. Joint effects of different human papillomaviruses and *Chlamydia trachomatis* infections on risk of squamous cell carcinoma of the cervix uteri. *Eur J Cancer* 2004;40:1058–65.

PUBLICATION IV

Association of Chlamydia trachomatis infection with cervical atypia in adolescent women with short- or long-term use of oral contraceptives: a longitudinal study in HPV vaccinated women.

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BMJ Open

Association of *Chlamydia trachomatis* infection with cervical atypia in adolescent women with short-term or long-term use of oral contraceptives: a longitudinal study in HPV vaccinated women

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ABSTRACT

Objective We assessed the relationship between *Chlamydia trachomatis* infection, duration of oral contraceptive (OC) use and cervical atypia among young adult Finnish women.

Design A longitudinal study.

Setting and participants Women who were included in this study participated in a community-randomised trial on the effectiveness of human papillomavirus (HPV) vaccination and *C. trachomatis* screening at ages 18.5 and 22 years in Finland. They completed questionnaires on both visits about sexual behaviours. The cytology test results at age 18.5 and 22 years were also available for those women. The total number of participants in this study at 18.5 years of age were 11 701 and at 22 years of age were 6618.

Main outcome measure ORs with 95% CIs using univariable and multivariable logistic regression were used to assess the association between *C. trachomatis* infection, duration of OC and squamous intraepithelial lesions (SIL).

Results There were 940 cytological SIL cases at the first screening visit and 129 cytological SIL cases at the second screening visit. Among the 22 years old, more than fourfold adjusted risk of SIL was associated with *C. trachomatis* positivity. The HPV16/18, condom use, smoking and number of sexual partners adjusted joint effect of prolonged OC use and *C. trachomatis* was significantly increased (OR 4.7, 95% CI 1.7 to 12.8) in the 22-year-old women. This observed joint effect was 1.6 times higher than expected on a multiplicative scale. On additive scale, the observed relative excess risk from interaction was 1.8.

Conclusion The risk of SIL in HPV vaccinated women is significantly increased if they are *C. trachomatis* positive and have used OC for 5 or more years. The biological basis may be lack of condom facilitated protection against sexually transmitted diseases.

Trial registration number NCT00534638.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The longitudinal study design allows the prospective evaluation of oral contraceptive use.
⇒ The well-controlled sensitive testing of *Chlamydia trachomatis* by PCR.
⇒ The inadequate data on barrier methods of contraception might have confounded the results.

BACKGROUND

Chlamydia trachomatis infection is the most common sexually transmitted bacterial infection characterised by persistent inflammation of epithelial tissue and chronic, also neoplastic, disease sequelae.[1] *C. trachomatis* is especially common in sexually active young women with early age at first intercourse, multiple sex partners and use of non-barrier contraceptive methods.[2] Most notably, *C. trachomatis* infection is associated with the persistence of oncogenic human papillomavirus (HPV) infection.[3] Like smoking, *C. trachomatis* infection is a cervical carcinogenesis cofactor possibly independent of HPV.[4 5] On the other hand, concomitant infection with *C. trachomatis* and HPV types 18 or 45 is associated with synergistically increased risk of cervical intraepithelial neoplasia grade 3 (CIN 3), that is, high-grade squamous intraepithelial lesions (HSIL).[6]

The association between *C. trachomatis* infection and cervical neoplasia could be the result of confounding by overlapping HPV exposure and/or oral contraceptive (OC) use.[7–10] While the role of *C. trachomatis* and HPV infections in cervical carcinogenesis has been documented,[6–8] the interplay of *C. trachomatis* infection and duration of OC use has not been studied over time.

In this study, we have evaluated the risk of cytological SIL associated with the duration

of OC use among *C. trachomatis* positive and negative women. We have studied the joint effect of *C. trachomatis* infection and duration of OC use on the development of cytological SIL in a large community-randomised trial cohort followed-up for up to 10 years.[11]

MATERIAL AND METHODS

Study conduct

The study material was obtained from the community-randomised trial on the effectiveness of gender-neutral or girls-only HPV vaccination strategies conducted in Finland.[11 12] In 2007–2009, all 80 272 Finnish boys and girls (1992–1995 birth cohorts) resident in 33 Finnish communities were identified using Finnish Population Register in three study arms each with 11 communities. All the males and females received either Cervarix (HPV16/18) vaccine (90%) or Engerix (hepatitis B-virus, HBV) vaccine (10%) in arm A. All the females received either HPV vaccine (90%) or HBV vaccine (10%) and the males received HBV vaccine in arm B. In arm C all the males and females received the HBV vaccine. Virtually all 99.4% of the participants received all three vaccine doses at months 0, 1 and 6.[13]

In 2010–2014, all the 1992–1995 born female residents in the trial communities were invited at the age of 18.5 years for a follow-up visit. They were offered cross-vaccination with either HPV16/18-vaccine or HBV-vaccine, if they had not received them earlier in the trial. Cervical cytological sample taken by a study nurse and a self-collected cervico-vaginal sample for HPV and/or *C. trachomatis* testing were obtained.[13] All the female participants during the follow-up agreed to participate in a *C. trachomatis* screening trial and filled in a questionnaire on demographics, life habits and sexual behaviour.[13]

Four years later (2014–2018) all the HPV16/18 vaccinated female participants were invited for the second follow-up visit at age 22 years. Again, cervical cytological sample and a self-collected cervico-vaginal sample for HPV and/or *C. trachomatis* testing were obtained, and the participants also filled in the questionnaires.[13]

In this study, we have four different types of datasets at both 18.5 years and 22 years of age: questionnaire dataset, *C. trachomatis* dataset, cytology dataset and HPV DNA dataset. All the four datasets were merged one-by-one at a time. All those which did not match/merge with any of the datasets were excluded from the study at the end (figure 1) of merging. The total number of study participants after merging all these datasets in this study at 18.5 years of age were 11 701 and at 22 years of age were 6618. The women were included if they merged with at least one or all the datasets. That is also the reason not all the merged women have complete data. However, during the analysis, complete case approach was applied and none of the missing values

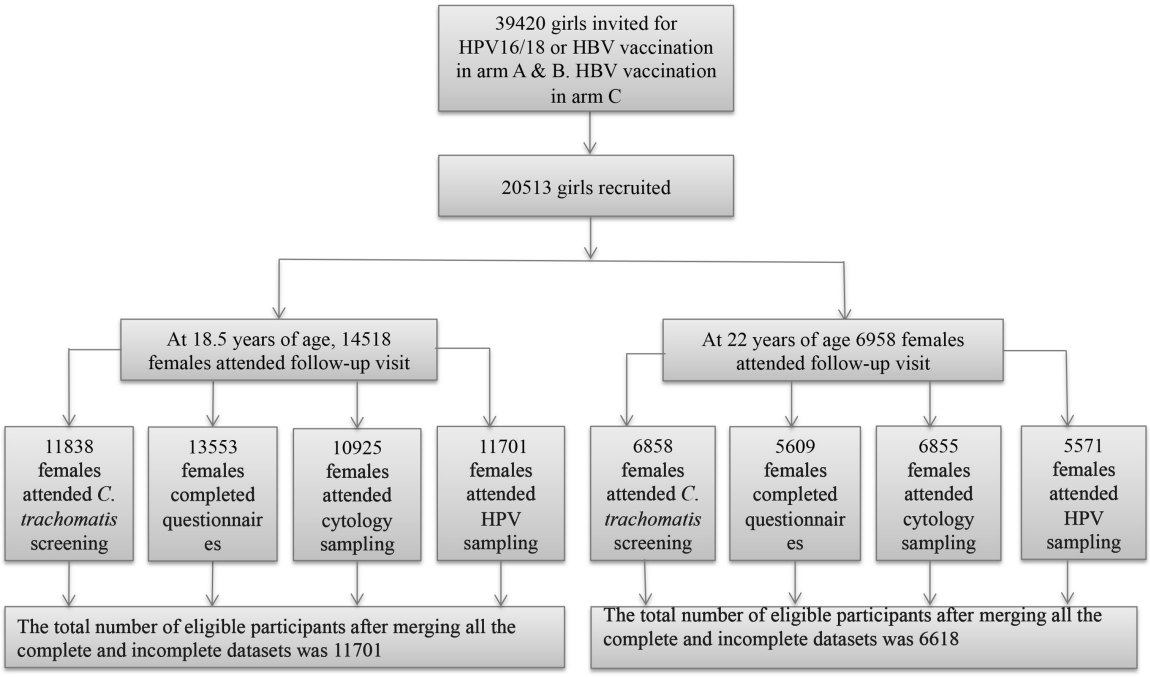

Figure 1 Flow diagram of female community randomised trial participants. 1852 participants at 18.5 years and 340 participants at 22 years of age were excluded from the study while merging the datasets as they did not merge with any of the other datasets. HBV, hepatitis B-virus; HPV, human papillomavirus.

were included neither in adjusted nor in unadjusted analyses.

Patient and public involvement

The study presents analysis of secondary data. There was no patient and public involvement.

Laboratory analysis

The self-collected cervical samples were analysed for the presence of HPV-DNA and further identification of the detected HPV types 6/11/16/18/31/33/35/39/45/51/52/56/58/59/66/68 by PCR (MGP primers) and matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry.[14] The DNA sequence specific for the presence of *C. trachomatis* in the sample was detected by commercial PCR (Abbott-TM).

Statistical analyses

The main independent variables of the study were *C. trachomatis* status at ages 18.5 and 22 years, and duration of OC use (≤2 years and >2 years at 18.5 years of age, ≤5 years and >5 years at 22 years of age). Information about OC use was available from the questionnaires. Years since start of OC use was calculated using questionnaire information about years between start of OC use and sexual debut and information about age at sexual debut.[15] The endpoint was cytological SIL divided into low-grade SIL and HSIL. The questionnaire dataset, *C. trachomatis* result dataset, HPV and cytology dataset were merged using personal ID.

ORs with 95% CI were calculated using logistic regression models to assess the risk of SIL associated with the *C. trachomatis* infection at age 18.5 and 22 years. In this study, we have interpreted OR as relative risk due to rare events. The risk estimates were adjusted to account for the potential confounding due to HPV DNA, condom use, number of sexual partners and smoking using multivariable logistic regression. Finally, the joint effect of duration of OC use and *C. trachomatis* was calculated and adjusted for potential confounders. In the main analysis, women with missing values on confounders were excluded from all regression models to ensure that the model included the same women (complete case analysis). As a sensitivity analysis, we repeated the crude analysis including women with missing values on confounders, to see whether this changed our estimates. All analyses were done by using Stata V.14.0 (Stata Corp: release 14).

RESULTS

At the first visit, the total number of cytological SIL cases was 940. The cytological results were missing for 781 participants out of 11 701 of the study participants (table 1). The number of cytological HSIL cases was 36 at 18.5 years of age. The baseline characteristics of 940 women with cytological SIL and 9980 women without cytological SIL were comparable. The age at first sexual intercourse, number of new sexual partners and OC use were materially similar in the SIL cases and healthy controls at the first visit (table 1). There were no notable differences in HPV16/18 positivity. Also smoking and condom use were comparable at 18.5 years of age (table 1).

At the second visit, the total number of cytological SIL cases was 129 of 6618 (1.9%) of the study participants (table 1). The number of cytological HSIL cases was 27 at 22 years of age. At the second visit, while condom use did not differ between the 129 SIL cases and 6489 healthy controls, smoking and number of new sexual partners were somewhat higher in the SIL cases (table 1). There were double the SIL cases with three or more sexual partners compared with healthy controls. OC use was comparable between SIL cases and the healthy controls (table 1).

In the univariable analysis, the risk of cytological SIL associated with *C. trachomatis* was slightly, although non-significantly increased (OR 1.1) at 18.5 years of age (table 2). There was no further risk after adjusting for HPV16/18, condom use last year, smoking and number of sexual partners (table 2). At 22 years of age, the crude risk of SIL was highly significantly increased (OR 4.6, 95% CI 2.6 to 8.3) in *C. trachomatis* positive women compared with *C. trachomatis* negative women (table 2). In the multivariable analysis, the adjusted OR was still significantly high (OR 4.3, 95% CI 2.2 to 8.5) among the *C. trachomatis* positive women (table 2). When we repeated the crude regression analysis including women with missing values on confounders, the results were virtually unchanged.

Finally, the joint effect of *C. trachomatis* and duration of OC use was assessed. Among the 18.5 years old, the joint effect of *C. trachomatis* and duration of OC use on SIL was OR 0.9 (95% CI 0.4 to 1.7). The separate effects of *C. trachomatis* and duration of OC use were insignificant (table 3). The risk estimates were adjusted for HPV16/18, condom use last year, smoking and number of sexual partners. The missing values were not included in the analyses.

On the contrary, in the 22 years old, even after adjusting for the potential confounders (HPV16/18, condom use last year, smoking and number of sexual partners) the joint effect risk of cytological SIL was (OR 4.7, 95% CI 1.7 to 12.8) in *C. trachomatis* positive women who had used OC for 5 or more than 5 years compared with *C. trachomatis* negative and short-term OC users (table 4). The individual adjusted effects of *C. trachomatis* and duration of OC use on SIL were (OR 2.9, 95% CI 0.8 to 10.3) and (OR 1.0, 95% CI 0.5 to 1.9), respectively. Under a multiplicative model, the expected joint effect of *C. trachomatis* positivity and five or more than 5 years of OC use was (OR 2.9, 95% CI 0.6 to 14.0). The observed joint effect of 4.7 was 1.6 times higher than expected on a multiplicative scale. Under an additive scale, the relative excess risk from interaction was (OR 1.8, 95% CI −3.5 to 7.2). The missing values were not included in the analyses.

Table 1 Cohort characteristics of study endpoints at 18.5 and at 22 years of age

	At 18.5 years		At 22 years	
Categories	**SIL (N=940)**	**No SIL (N=9980)**	**SIL (N=129)**	**No SIL (N=6489)**
Age at first sexual intercourse				
8–13 years	31 (3.3%)	316 (3.2%)	2 (1.5%)	162 (2.5%)
14–18 years	712 (75.7%)	7546 (75.6%)	85 (65.9%)	4392 (67.7%)
19–22 years	NA	NA	13 (10.1%)	697 (10.7%)
Missing	197 (21.0%)	2118 (21.2%)	29 (22.5%)	1238 (19.1%)
New sexual partners last year (n)				
2 or less	546 (58.0%)	6068 (60.8%)	61 (47.3%)	4209 (64.9%)
3 or more	197 (21.0%)	1793 (18.0%)	39 (30.2%)	1032 (15.9%)
Missing	197 (21.0%)	2119 (21.2%)	29 (22.5%)	1248 (19.2%)
Current smoking				
No	579 (61.6%)	5971 (59.8%)	49 (38.0%)	3273 (50.4%)
Yes	297 (31.6%)	3203 (32.1%)	38 (29.5%)	1518 (23.4%)
Missing	64 (6.8%)	806 (8.1%)	42 (32.5%)	1698 (26.2%)
OC-use				
No	152 (16.2%)	1645 (16.5%)	14 (10.8%)	573 (8.8%)
Yes	591 (62.8%)	6202 (62.1%)	85 (65.9%)	4648 (71.6%)
Missing	197 (21.0%)	2133 (21.4%)	30 (23.3%)	1268 (19.6%)
Condom use last year				
Not at all	182 (19.4%)	1978 (19.8%)	32 (24.8%)	1965 (30.3%)
Sometimes	207 (22.0%)	2216 (22.2%)	24 (18.6%)	1164 (17.9%)
In half of the intercourse	95 (10.1%)	985 (9.90%)	12 (9.3%)	603 (9.3%)
Almost always	135 (14.4%)	1160 (11.6%)	21 (16.3%)	669 (10.3%)
Always	111 (11.8%)	1433 (14.4%)	10 (7.7%)	776 (12.0%)
Missing	210 (22.3%)	2208 (22.1%)	30 (23.3%)	1312 (20.2%)
HPV16/18				
Negative	886 (94.3%)	9500 (95.2%)	106 (82.2%)	5307 (81.7%)
Positive	54 (5.70%)	480 (4.80%)	2 (1.5%)	49 (0.8%)
Missing*			21 (16.3%)	1133 (17.5%)

*Cytological results were missing for 781 of the HPV16/18 results among 18.5 years old.
HPV, human papillomavirus; OC, oral contraceptive; SIL, squamous intraepithelial lesions.

Table 2 Risk of cervical cytological squamous intraepithelial neoplasia by *C. trachomatis* at 18.5 and 22 years old

		SIL		Adjusted SIL*
Category	**N**	**n**	**OR (95% CI)**	**OR (95% CI)**
At 18.5 years				
Chlamydia seronegative women	10 512	901	1	1
Chlamydia seropositive women	408	39	1.1 (0.8 to 1.6)	1.0 (0.6 to 1.5)
At 22 years				
Chlamydia seronegative women	5352	86	1	1
Chlamydia seropositive women	198	14	4.6 (2.6 to 8.3)	4.3 (2.2 to 8.5)

N is the number of *C. trachomatis* in each age group. n is number of SIL cases.
*Adjusted for HPV16/18, condom use last year, smoking and number of sexual partners.
HPV, human papillomavirus; SIL, squamous intraepithelial lesions.

Table 3 Risk of cervical cytological squamous intraepithelial neoplasia (SIL) by joint effect of duration of oral contraceptive (OC) use and *C. trachomatis* positivity at 18.5 years

			SIL		Adjusted SIL*
Risk factors		**N**	**n**	**OR (95% CI)**	**OR (95% CI)**
CT#	Duration of OC use				
0	0	2746	233	1	1
0	1	3675	298	0.9 (0.8 to 1.1)	0.9 (0.7 to 1.1)
1	0	88	7	0.9 (0.4 to 1.9)	0.7 (0.3 to 1.7)
1	1	126	11	1.0 (0.5 to 1.8)	0.9 (0.4 to 1.7)

In duration (0=5 or less than 5 years/short-term OC use, 1= more than 5 years/long-term OC use).
*Adjusted for HPV16/18, condom use last year, smoking and number of sexual partners.
CT, *C. trachomatis* (0= CT negative, 1=CT positive); HPV, human papillomavirus.

DISCUSSION

This study shows that *C. trachomatis* positive HPV-vaccinated women have increased risk of cytological SIL. The adjusted risk of SIL associated with *C. trachomatis* positivity was significantly higher (4.3-fold) even after adjusting for the potential confounders among 22-year-old women. The observed joint effect of *C. trachomatis* positivity and long-term duration of OC use (more than 5 years) was higher than expected on both multiplicative and additive scale compared with *C. trachomatis* negative and short-term OC users (5 or less than 5 years) of OC use among 22-year-old women. The observed synergistic interaction was an unanticipated finding in this study.

In a number of other prospective studies, *C. trachomatis* has been proven to set the stage for cervical carcinogenesis leading to CIN3, possibly even independently of HPV.[5–10] Our finding that *C. trachomatis* was associated with the increased risk of SIL in HPV-vaccinated women is in line with these studies since *Chlamydia* probably interacts also with non-vaccine HPV types. *C. trachomatis* is most common in adolescents and young adults of age 15–29,[16] which is also the case in our study. The reason for increased susceptibility to sexually transmitted infections is because the young adults are more into casual sex and often do not use barrier methods of contraception.[17] This was also the case in our study, where most of the participants replied infrequent and less use of condom as well as having multiple sexual partners which predisposes to *Chlamydia* associated carcinogenesis.[18]

In our study, the mean age at first sexual intercourse was at 16 years of age. While OCs are one of the most common contraceptive methods among adolescents, they provide protection against unwanted pregnancies, but not against the sexually transmitted infections.[16] There are several studies showing an increased risk of *C. trachomatis* infection associated with OC use.[19–21] This is also in line with our findings. There is biological plausibility to assume synergistic interaction between OC use and cervical *C. trachomatis* infection. The long-term use of OC may increase the growth and persistence of *C. trachomatis* infection by altering the immune response especially among those, who do not use only barrier method of contraception.[19] *C. trachomatis* infection also favours the persistence of all (both vaccine and non-vaccine) high-risk HPV types which facilitates progress of neoplastic lesions.[18] Also, there are epidemiological studies which have found that the risk of cervical neoplasia/cancer increases with the increase in duration of OC use.[22 23] Thus, both the *C. trachomatis* infection and long-term OC use are associated with the increased risk of cervical neoplasia, which is supported by our joint effect risk of long-term OC use and *C. trachomatis* infection.

Table 4 Risk of cervical cytological squamous intraepithelial neoplasia (SIL) by joint effect of duration of oral contraceptive (OC) use and *C. trachomatis* positivity at 22 years

			SIL		Adjusted SIL*
Risk Factors		**N**	**n**	**OR (95% CI)**	**OR (95% CI)**
CT#	Duration of OC use				
0	0	2293	30	1	1
0	1	1958	36	1.4 (0.9 to 2.3)	1.0 (0.5 to 1.9)
1	0	79	3	3.0 (0.9 to 9.9)	2.9 (0.8 to 10.3)
1	1	90	8	7.3 (3.3 to 16.6)	4.7 (1.7 to 12.8)

In duration (0=5 or less than 5 years/short-term OC use, 1= more than 5 years/long-term OC use).
*Adjusted for HPV16/18, condom use last year, smoking and no. of sexual partners.
CT, *C. trachomatis* (0= CT negative, 1=CT positive); HPV, human papillomavirus.

The main strength of our study is the large study population and longitudinal study design, which allowed the over time evaluation of OC use and timely measurement (and treatment) of the *C. trachomatis* infection. Another strength is the well-controlled and sensitive testing of *C. trachomatis* by PCR.

One limitation of our study is the number of cytological HSIL finding is small. Another limitation of our study is inadequate data on the use of barrier methods of contraception, which might have confounded the results. Yet another limitation of our study could be, that we have not considered the missing values in tables 2 and 3, which might bias the results. However, we checked including the missing values but the estimates did not differ much. Furthermore, we do not have information about the types of OC used. The risk might differ among the various types of OC depending on the hormonal composition.

In conclusion, we found increased risk of SIL associated with *C. trachomatis* positivity in HPV vaccinated population. Although based on small number of cases, the joint effect of *C. trachomatis* positivity and long-term use of OC on risk of SIL was higher than effects expected on the basis of additive or multiplicative interaction, which suggests synergism between the two variables. People who use OC as a means of contraception, may not use barrier methods of contraception and hence are prone to many sexually transmitted diseases. Thus, contraceptive and sexual health counselling should be enforced also among HPV vaccinated young women using oral/hormonal contraceptives. Additional studies are required to understand the biological basis of the interaction effect better.

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Contributors IA developed the research protocol, analysed the data, prepared the manuscript, revised and submitted the manuscript. TE contributed in data acquisition and data interpretation. KH and MH collected cervical cytological samples. PN made the cytological diagnoses and helped in the revision of the paper. TL commented on the draft of the paper and helped in the revision of the manuscript. DA commented on the draft of the paper. ML helped in data acquisition, contributed in the development of research plan, analysis plan, commented on the draft of the paper and the revision of the paper. ML is also an author acting as guarantor.

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Data availability statement Data available upon reasonable request, with appropriate ethics approval.

Addendum "Data availability statement " Data available upon reasonable request, with appropriate ethics approval.In Figure 1, all the red colored texts and numbers need to be changed into black. thank you.

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REFERENCES

1 Paavonen J, Turzanski Fortner R, Lehtinen M, *et al*. Chlamydia trachomatis, pelvic inflammatory disease, and epithelial ovarian cancer. *J Infect Dis*. In Press 2021;224:S121–7.
2 Handsfield HH, Jasman LL, Roberts PL, *et al*. Criteria for selective screening for Chlamydia trachomatis infection in women attending family planning clinics. *JAMA* 1986;255:1730–4.
3 Silins I, Ryd W, Strand A, *et al*. Chlamydia trachomatis infection and persistence of human papillomavirus. *Int J Cancer* 2005;116:110–5.
4 Kapeu AS, Luostarinen T, Jellum E, *et al*. Is smoking an independent risk factor for invasive cervical cancer? A nested case-control study within Nordic biobanks. *Am J Epidemiol* 2009;169:480–8.
5 Lehtinen M, Ault KA, Lyytikainen E, *et al*. Chlamydia trachomatis infection and risk of cervical intraepithelial neoplasia. *Sex Transm Infect* 2011;87:372–6.
6 Luostarinen T, Namujju PB, Merikukka M, *et al*. Order of HPV/Chlamydia infections and cervical high-grade precancer risk: a case-cohort study. *Int J Cancer* 2013;133:1756–9.
7 Wallin K-L, Wiklund F, Luostarinen T, *et al*. A population-based prospective study of Chlamydia trachomatis infection and cervical carcinoma. *Int J Cancer* 2002;101:371–4.
8 Anttila T, Saikku P, Koskela P, *et al*. Serotypes of Chlamydia trachomatis and risk for development of cervical squamous cell carcinoma. *JAMA* 2001;285:47–51.
9 Smith JS, Bosetti C, Muñoz N, *et al*. Chlamydia trachomatis and invasive cervical cancer: a pooled analysis of the IARC multicentric case-control study. *Int J Cancer* 2004;111:431–9.
10 , Appleby P, Beral V, *et al*, International Collaboration of Epidemiological Studies of Cervical Cancer. Cervical cancer and hormonal contraceptives: collaborative reanalysis of individual data for 16,573 women with cervical cancer and 35,509 women without cervical cancer from 24 epidemiological studies. *Lancet* 2007;370:1609–21.
11 Lehtinen M, Apter D, Baussano I, Iacopo B, *et al*. Characteristics of a cluster-randomized phase IV human papillomavirus vaccination effectiveness trial. *Vaccine* 2015;33:1284–90.
12 Lehtinen M, Söderlund-Strand A, Vänskä S, *et al*. Impact of gender-neutral or girls-only vaccination against human papillomavirus-Results of a community-randomized clinical trial (I). *Int J Cancer* 2018;142:949–58.
13 Lehtinen M, Luostarinen T, Vänskä S, *et al*. Gender-Neutral vaccination provides improved control of human papillomavirus types 18/31/33/35 through herd immunity: results of a community randomized trial (III). *Int J Cancer* 2018;143:2299–310.
14 Louvanto K, Eriksson T, Gray P, *et al*. Baseline findings and safety of infrequent vs. frequent screening of human papillomavirus vaccinated women. *Int J Cancer* 2020;147:440–7.
15 Adhikari I, Eriksson T, Luostarinen T, *et al*. The risk of cervical atypia in oral contraceptive users. *Eur J Contracept Reprod Health Care* 2018;23:12–17.
16 Halvarsson V, Ström S, Liljeros F. The prescription of oral contraceptives and its relation to the incidence of Chlamydia and abortion in Sweden 1997-2005. *Scand J Public Health* 2012;40:85–91.
17 Nikula M, Koponen P, Haavio-Mannila E, *et al*. Sexual health among young adults in Finland: assessing risk and protective

behaviour through a general health survey. *Scand J Public Health* 2007;35:298–305.
18 Paavonen J. *Chlamydia trachomatis* and cancer. *Sex Transm Infect* 2001;77:154–6.
19 Baeten JM, Nyange PM, Richardson BA, *et al*. Hormonal contraception and risk of sexually transmitted disease acquisition: results from a prospective study. *Am J Obstet Gynecol* 2001;185:380–5.
20 Cottingham J, Hunter D. Chlamydia trachomatis and oral contraceptive use: a quantitative review. *Genitourin Med* 1992;68:209–16.
21 Peipert JF, Lapane KL, Allsworth JE, *et al*. Women at risk for sexually transmitted diseases: correlates of intercourse without barrier contraception. *Am J Obstet Gynecol* 2007;197:474.e1–474.e8.
22 Smith JS, Green J, Berrington de Gonzalez A, *et al*. Cervical cancer and use of hormonal contraceptives: a systematic review. *Lancet* 2003;361:1159–67.
23 Moreno V, Bosch FX, Muñoz N, *et al*. Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. *Lancet* 2002;359:1085–92.