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Interval between menarche and the start of sexual activity may not be associated with the risk of cervical atypia

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ABSTRACT

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

Methods A total of 4808 (16-17 year-old) women were enrolled in the PATRICIA trial and randomized to receive human papillomavirus (HPV) 16/18 vaccine or hepatitis A-virus vaccine in 2004-2005. The association of cervical atypia and time interval between the FSI or the start of OC use, and menarche was assessed in the control group who had participated semi-annual clinical follow-up visits with cervical *Chlamydia trachomatis*, HPV DNA and cytology testing for four years. Altogether 914 women had normal baseline cervical cytology and answered behavioural questionnaires at enrolment and end of the follow-up.

Results The mean ages at menarche, at the FSI and at the start of OC use were 12.4, 16.0 and 16.4. *C. trachomatis*infection was associated with an increased risk of cervical atypia in women with a short (<3 years) interval between menarche and the FSI/start of OC use (odds ratio, OR 2.0 95% confidence interval [CI] 1.0-3.9, and OR 2.2 95%CI 1.0-5.1) whereas HPV16/18 infection was associated with increased atypia risk estimates in women with a longer (≥3 years) interval. In women with a short interval between menarche and the FSI, early age at the start of OC use was not associated with an increased risk of cervical atypia neither in univariate (OR 0.7) nor in multivariable analyses (OR 0.6; 95%CI 0.3-1.2).

Conclusion Short interval between menarche and age at the start of oral contraceptive use does not increase the risk of cervical atypia.

Key words cervical neoplasia, *Chlamydia trachomatis*, first sexual intercourse, human papillomavirus, menarche, oral contraceptives

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STRENGTHS AND LIMITATIONS OF THIS STUDY:

- The association of interval between menarche and age at the start of sexual activity with cervical atypia assessed by various indicators
- A large HPV vaccination trial cohort with standardized clinical and laboratory procedures
- The repeated self-reported study questionnaires were less subject to recall bias
- Use of the overall cervical atypia end-point might dilute the effects

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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, squamous intraepithelial lesions (SIL) 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], life-time number of sexual partners,[8], age at the first sexual intercourse (FSI),[9], parity,[10] and use of oral contraceptives,[11].

Both early age at the first sexual intercourse and early age at the start of oral contraceptive (OC) use are associated with an increased risk of SIL and CIN,[9, 12-14]. Furthermore, short lag between menarche and the 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 the oncogenic HPV types are established more readily in the immature cervix,[13, 15]. However, whether or not early start of OC use has an independent role here is open. The interplay of time interval between age at the start of OC use or age at the 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 the short interval between menarche and age at the start of OC use or age at the FSI.

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MATERIALS AND METHODS

Study Sample

The study population consists of women enrolled in the control arm of a double-blinded, multi-national randomized controlled PATRICIA trial (registration number NCT00122681) whose primary aim was to evaluate the vaccine efficacy (VE) of HPV16/18 vaccine against CIN2+,[16, 17]. Full descriptions 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 (2,409 received at least one dose of HPV16/18 vaccine) and 2,399 women (received at least one dose of hepatitis A-virus (HAV) vaccine). The criteria of having no more than 6 lifetime sexual partners was not applied in Finland, so all women interested and willing to participate the study were included,[18].In Finland, the trial was approved by the Finnish national ethics committee (TUKIJA 1174/04) in 2004.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 year-old when exiting the trial were sent a questionnaire on living conditions, life-habits and sexual health. All women (914) who had received the HAV-vaccine, had baseline negative cervical cytology and answered the questionnaires both at enrolment and at the end of the follow-up were eligible (Table 1).

Data Collection

The questionnaires collected information in addition to living-conditions and life-habits about the history of OC-use, other contraceptives use, smoking, menarche and sexual habits. The end-of study questionnaire was most complete as for start of sexual habits, and was used in the analysis. The age

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at the start of OC use, menarche and age at the FSI were the independent variables in this study. An interval of less than 3 years and more than or equal to 3 years was calculated between menarche and age at the start of OC use as well as between menarche and FSI. Data on smoking ('never-smokers', 'past smokers' and 'present smokers'), life-time number of sexual partners ('none', '1', '2-4', '5-9' and 'more than 10'), condom use ('non-user', 'user' and 'do not know') and sexually transmitted infections (HPV16/18 and *C. trachomatis*) were used as co-variables.

Laboratory analysis and endpoints

In the PATRICIA trial, semi-annual cervical cytological and DNA samples were obtained in conjunction of pelvic examination. PCR analyses for C. trachomatis and HPV DNA were performed as described,[18].During the follow-up 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 cervical intraepithelial lesions (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 more 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 more than 3 years lag between menarche and OC use.

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Patient and Public Involvement

The attitudes to and willingness to participate a HPV vaccination trial were evaluated in a questionnaire sent to adolescents in one of the major study site communities,[19]. Launching the HPV16/18 vaccination trial without sexual partner number exclusion criteria was a deliberate decision to guarantee the population-based nature of the trial,[17]. The birth cohorts Q2-Q41986-Q11988 were invited and information lectures about the trial were arranged in secondary high-schools during the school-year 2004-2005.

Statistical analysis

The outcome variables were analysed in the univariate and multivariable logistic regression models along with the independent variables and above listed co-variates. The risks are reported as the odds ratios (OR) with95% confidence interval (CI). The statistical analysis was performed using Stata version 14.0 (Stata Corp LP, Statistical Software: Release 14. College Station, TX, USA).

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RESULTS

Baseline characteristics of our study cohort attending semiannual follow-up visits for four years are materially homogeneous with little variation (Table 1).

Table 1. Characteristics of young adult women (N=914) who attended eight semi-annual follow-up visits and a subgroup of these women (n=198) who developed cervical atypia during the four years of follow-up by the average age of 23 years

Characteristics	Attendees N=914	%	Women with atypia n=198	%
Age				
22	422	46.2	94	47.5
23	490	53.6	104	52.5
24	2	0.2	0	0
Missing	0	0	0	0
Age at menarche				
≤11	194	21.2	52	26.3
12-14	659	72.1	136	68.7
≥15	53	5.8	7	3.5
Missing	8	0.9	3	1.5
Age at first intercourse				
12-16	602	65.9	129	65.2
17-22	274	30.0	61	30.8
Missing	38	4.2	8	4.0
LNSP*				
0	3	0.3	1	0.5
1	131	14.3	29	14.6
2-4	283	31.0	57	28.8
5-9	236	25.8	50	25.3
≥10	231	25.2	56	28.3
Missing	30	3.3	5	2.5
Oral contraceptive use				
Non -user	62	6.8	15	7.6
User	843	92.2	180	91.0
Missing	9	1.0	3	1.5
Age at start of OC use				
12-16	504	55.1	104	52.5
17-22	372	40.7	86	43.4
Missing	38	4.2	8	4.0
Condom use				
Non -user	415	45.4	98	49.5
User	406	44.4	83	42.0
Don't know	76	8.3	16	8.1
Missing	17	1.9	1	0.5
Smoking				
Never	526	57.5	109	55.0
Past	93	10.2	16	8.1
Present	291	31.8	73	36.9
Missing	4	0.4	0	0

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HPV16				
Negative	712	78.0	146	73.7
Positive	201	22.0	52	26.3
Missing	1	0.1	0	0
HPV18				
Negative	793	86.8	166	83.8
Positive	120	13.1	32	16.2
Missing	1	0.1	0	0
Chlamydia				
Negative	811	88.7	175	88.4
Positive	103	11.3	23	11.6
Missing	0	0	0	0

*LNSP= life-time number of sexual partners
#HPV16, HPV18 and *C.trachomatis* infections were recorded semi-annually for 4 years.

Age at menarche was between 12 and 14 years for 659 (72.1%) 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 (55.1%) participants, respectively.

By the end of the follow-up period, 198 (21.7%) of 914 women were identified with cervical atypia (Table 1). Almost one third of the women with cervical atypia (56 of 198, 28.3%) had had more than 10 sexual partners. Half of the women with or without cervical atypia (49.5%) and (45.4%) did not regularly use condoms. Most of the women (180 of 198, 91.0%) with cervical atypia had used oral contraceptives. Age at the start of OC use for a majority of these women (104 out of 198, 52.5%) was between 12 and 16 years (Table 1).

During the four year follow-up 201 (22%) of all women tested positive for HPV16 and 120 (13.1%) tested positive for HPV18 (Table 1). One third of women with either HPV16 or HPV18, or both were diagnosed with cervical atypia during the follow-up. The number of women testing positive for *C. trachomatis*was 103 (11.3%), and the number of *C. trachomatis* positive women with cervical atypia was 23 (11.6%) (Table1).

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We categorized 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 the FSI using a stratification of <3 years and ≥3 years (Table 2).

Table 2. Distribution of cervical atypia risk factors by interval between menarche and age at the start of oral contraceptive(OC) use or between menarche and age at the first sexual intercourse (FSI) in young adult women followed up for 4 years

Characteristics	Interval between menarche and age at the start of oral contraceptive use		Interval between menarche and age at the first sexual intercourse	
	Interval <3 yrs. (N=192)	Interval ≥3 yrs. (N=676)	Interval <3 yrs. (N=303)	Interval ≥3 yrs. (N=565)
	pos/mean (%)/[SD]	pos/mean (%)/[SD]	pos/mean (%)/[SD]	pos/mean (%)/[SD]
C. trachomatis	39 (20.3)	60 (8.9)	55 (18.2)	44 (7.8)
HPV 16	55 (28.6)	142 (21.0)	97 (32.1)	100 (17.7)
HPV 18	35 (18.2)	83 (12.3)	47 (15.6)	71 (12.5)
HPV 16/18	69 (35.9)	190 (28.1)	115 (38.1)	144 (25.4)
Smoking				
Never	84 (43.7)	406 (60.4)	135 (45.0)	356 (63.0)
Past smoker	22 (11.5)	69 (10.3)	37 (12.3)	54 (9.6)
Present smoker	86 (44.8)	197 (29.3)	128 (42.7)	155 (27.4)
Age at menarche	13.3 [1.2]	12.1 (1.1)	13.0 (1.3)	12.0 (1.1)
Age at the FSI	14.7 []1.2]	16.3 (1.9)	14.5 (1.2)	16.7 (1.7)
Age at the start of OC use	14.9[(1.29	16.8 (1.7)	15.3 (1.3)	17.0 (1.7)

The mean ages at menarche, at the 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 HPV16 positive than women in the ≥3 years interval categories (Table 2). The percentages of women with multiple (>5) life-time sexual partners were also higher in the short interval categories (Table 2).

In the univariate analysis, the risk of cervical atypia was evaluated separately in the short and long interval categories (Table 3).

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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, CIN) stratified by the interval between menarche (M) and age at the first sexual intercourse (FSI) or between menarche and age at the 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							
	M to 1st intercourse <3 years SIL/CIN1+		M to 1st intercourse ≥3 years SIL/CIN1+		M to start of OC use <3 years SIL/CIN1+		M to start of OC use ≥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	21/155	1.0 (0.6-1.8)	45/144	1.8 (1.1-2.7)	13/69	1.4 (0.6-3.0)	53/190	1.4 (1.0-2.1)
Negative	34/187	1	87/422	1	18/123	1	103/485	1
Chlamydia								
Positive	15/55	2.0 (1.0-3.9)	8/44	0.7 (0.3-1.6)	10/39	2.2 (1.0-5.1)	13/60	0.9 (0.5-1.7)
Negative	40/248	1	124/522	1	21/153	1	143/616	1
Smoking								
Yes	34/165	1.4 (0.8-2.6)	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	21/135	1	80/356	1	9/84	1	92/406	1
Condom use								
Yes	22/146	0.7 (0.4-1.2)	59/256	0.9 (0.7-1.2)	15/102	0.6 (0.3-1.2)	66/300	0.9 (0.7-1.2)
No	30/140	1	61/251	1	16/80	1	75/310	1
LNSP								
High	40/216	1.1 (0.6-2.1)	64/245	1.3 (0.9-2.0)	24/135	1.5 (0.6-3.8)	80/326	1.2 (0.8-1.7)
Low	15/87	1	68/321	1	7/57	1	76/350	1
Interval between age at the 1st intercourse and menarche								
<3 years	55/303	NA	0	NA	31/192	NA	24/111	0.9 (0.6-1.5)
≥3 years	0	NA	132/566	NA	0	NA	132/565	1
Interval between age at the start of OCs and menarche								
<3 years	31/192	0.7 (0.4-1.3)	0	NA	31/192	NA	0	NA
≥3 years	24/111	1	132/565	NA	0	NA	156/676	NA

*LNSP=lifetime number of sexual partner, high = 5 or more, low < 5, NA= not available

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Cervical atypia risk estimates associated with HPV16/18 were increased (OR 1.8, CI: 1.1-2.7 and OR 1.4, CI: 1.0-2.1) in the longer (≥3 years) interval categories. On the contrary, the cervical atypia risk associated with *C. trachomatis* was increased (OR 2.0, CI 1.0-3.9; OR 2.2, CI: 1.0-5.1) 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).The risk of cervical atypia associated with the short interval between menarche and age at the start of OC use was somewhat decreased (OR 0.7, CI: 0.4-1.3) when also the interval between menarche and age at the FSI was short (Table 3). Also, there was no risk of atypia associated with the long interval between menarche and the start of OC use (Table 3). The risk remained the same even when the interval was entered as a continuous variable into the model.

In a multivariable analysis, including all the above-mentioned variables, the corresponding OR did not materially differ from that of univariate analysis but the upper 95% confidence limits approached 1 (OR 0.6, CI: 0.3-1.1) (data not shown). Stepwise exclusion of one variable at a time from the multivariable model was performed to check the interdependency of interval between menarche, age at the start of OC use, and age at the FSI in this context. Exclusion of any of the above-mentioned variables did not affect significance of the estimates (data not shown).

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DISCUSSION

We found that cervical atypia was not associated with the early start of sexual activity after menarche. Increased risk of cervical atypia was associated with *C. trachomatis* shortly after menarche, and with HPV16/18 infections more than three years after menarche.

The absence of HPV16/18 associated risk of cervical atypia in women with short lag between menarche and 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, however, is in line with Collins et al. who reported that the increased interval between menarche and age at the first sexual intercourse increases the risk of HPV infection,[20]. On the other hand, it has been reported that when *C. trachomatis* infection precedes or virtually co-occurs with HPV infection the risk of cervical neoplasia associated with the joint infection is very high,[21] which seems to support our observations.

Our findings contradict with Ruiz et al. who first reported that short interval between menarche and age at the first sexual intercourse is a predictor of cervical cytological abnormalities and CIN [9]. While our homogeneous study population had ample power to detect a three-fold increased risk (Appendix) their study population was heterogeneous. Furthermore, we found lack of association between short interval of menarche and two measures of start of sexual activity (age at the first intercourse and age at the start of OC use). Overall, however, the different observations on the interval between menarche and start of sexual activity, and risk of cervical atypia [9, 12-14] reflect limited sample sizes.

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Our large HPV vaccination trial derived population of young adult women, standardized clinical and laboratory procedures are noteworthy. Furthermore, over entire follow-up period the trial participants received regular sexual health counseling which probably reduced possible confounding and bias in our study. To the best of our knowledge, the association between interval between menarche and age at the start of OC use with cervical atypia was now assessed for the first time.

Some limitations of our study are: Use of the overall cervical atypia end-point, which was necessary to retain 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 end-point questionnaire (at age 22) was, however, in line with the enrolment questionnaire (at age 18), e.g. for menarche. Moreover, questionnaire-based information on the sexual behavior is supposed to have adequate validity and reliability [22, 23]. In addition, the participants were distributed free contraceptives during the trial period, which might have affected the proportions of OC and condom users in our study.

In conclusion, while our study does not support the hypothesis that short interval between menarche and age at the start of sexual activity increases the risk of cervical atypia early age of acquiring *C. trachomatis* infections is setting the stage for cervical carcinogenesis.

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Footnotes

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

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Patient consent for publication: Not required

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Appendix. Power to find an association (p=0.05) for short interval between menarche and age at the start of sexual activity, and cervical atypia assuming up to 20% cumulative atypia incidence* and variable strength (odds ratio, OR) of the association in 900 women.

OR	**5%**	**10%**	**15%**	**20%**
0.5	0.22	0.49	0.72	0.87
2.0	0.64	0.90	0.98	1.00
4.0	1.00	1.00	1.00	1.00

*prevalence at the end of follow up

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Reported on page #
	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract -**longitudinal study**	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found- **We investigated whether the risk of cervical atypia is associated with a short interval between age at the first sexual intercourse (FSI) or age at the start of oral contraceptive (OC) use, and menarche. We found that a short interval between menarche and age at the start of oral contraceptive use do not increase the risk of cervical atypia.**	
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported- **Early age at the first sexual intercourse and early age at the start of oral contraceptive (OC) use are associated with an increased risk of SIL and CIN. The interplay of time interval between age at the start of OC use or age at the FSI, and menarche in cervical carcinogenesis has not been studied.**	1
Objectives	3	State specific objectives, including any prespecified hypotheses- **To investigate whether the risk of cervical atypia is associated with the short interval between menarche and age at the start of sexual activity.**	1
Methods			
Study design	4	Present key elements of study design early in the paper: **Longitudinal study including women (approx. 22 years) from the control arm (Hepatitis A vaccinated) of PATRICIA trial.**	2
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection-**The study was conducted in Finland. The study population consisted of women enrolled in the control arm of a double-blinded, multi-national randomized controlled PATRICIA trial. The women who had answered behavioral questionnaire after exiting from the trial in 2010 and were non-HPV vaccinated were enrolled in the present study.**	2
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up-**The participants had semi-annual follow-up when they were in the trial.**	3
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed- **NA**	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable-**Outcome cervical atypia**. **Exposure- menarche, age at**	3

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		first sexual intercourse and age at first use of oral contraceptive	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group-**Sexual and behavioural data were obtained from the questionnaire.**	2
Bias	9	Describe any efforts to address potential sources of bias- **The standardized clinical and laboratory procedures used in trial might have reduced the bias earlier in the study. Sexual health counselling of the participants during the follow-up also reduced the chances of bias.**	10
Study size	10	Explain how the study size was arrived at –**described above**	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why- **Quantitative variables such as age and number of sexual partners were categorised into different categories.**	2,3
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **NA**	
		(*b*) Describe any methods used to examine subgroups and interactions **NA**	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed- **potentially eligible 2098 women, after examining for eligibility 999, confirmed eligible and included in the study 914 women.**	2, 3
		(b) Give reasons for non-participation at each stage **Initially those who answered the questionnaire were all eligible. Later those who were HPV vaccinated were excluded. Finally the baseline cases were excluded.**	2
		(c) Consider use of a flow diagram **Flow diagram is presented in our earlier paper which is cited in this paper.**	2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders- **16-17 years old female from Finland were enrolled in the PATRICIA trial who were approximately 22 years old when exiting the trial.**	2
		(b) Indicate number of participants with missing data for each variable of interest-**Age at menarche 8, Age at first intercourse 38, Age at start of OC use 38. Shown in table 1**	5
		(c) Summarise follow-up time (eg, average and total amount) **4 years**	2
Outcome data	15*	Report numbers of outcome events or summary measures over time	**198 cervical atypia cases**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included-**0.9 (0.6-1.5) and 0.7 (0.4-1.3)**	8
		(*b*) Report category boundaries when continuous variables were	

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		categorized –**inclusive class interval**	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **NA**	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **NA**	
Discussion			
Key results	18	Summarise key results with reference to study objectives.**Increased risk of cervical atypia was found associated with *C. trachomatis* infection in women with short interval between menarche and start of sexual activity. Increased risk of cervical atypia was found associated with HPV16/18 infections in women with longer interval between menarche and start of sexual activity. Early start of OC-use may not increase the risk of atypia**	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias- **The questionnaire data were self-reported at the age of 22 years which is subject to recall bias.**	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence – **while our study does not support the hypothesis that short interval between menarche and age at the start of sexual activity increases the risk of cervical atypia early age of acquiring *C. trachomatis* infections is setting the stage for cervical carcinogenesis.**	11
Generalisability	21	Discuss the generalisability (external validity) of the study results- **This study is generalizable to the similar population of young women with more than 6 life time number of sexual partners as well as to those women who had an easy access to the contraceptive methods**.	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based-: **Matti Lehtinen and Dan Apter have grants from Merck & Co. Inc. and GSK for HPV vaccination trials through their employers (University of Tampere, ML; Family Federation Finland, DA)**	

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely

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available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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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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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 age at the first sexual intercourse (FSI) or age at the start of oral contraceptive (OC) use, and menarche.

Design A population-based cohort study.

Setting 16-17 year-old Finnish women 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 semi-annual clinical follow-up visits for four years. Altogether 913 women had normal baseline cervical cytology and answered behavioural questionnaires at enrolment and end of the follow-up.

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

Results The mean ages at menarche, at the FSI and at the start of OC use were 12.4, 16.0 and 16.4. *C. trachomatis* infection was associated with an increased risk of cervical atypia in women with a short (<3 years) interval between menarche and the FSI/start of OC use (odds ratio, OR 1.8 95% confidence interval [CI] 1.0-3.6, and OR 2.2 95% CI 1.0-5.1) whereas HPV16/18 infection was associated with increased atypia risk estimates in women with a longer (≥3 years) interval. In women with a short interval between menarche and the FSI, early age at the start of OC use was not associated with an increased risk of cervical atypia neither in univariate (OR 0.7) nor in multivariable analyses (OR 0.6; 95% CI 0.3-1.2).

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

Key words cervical neoplasia, *Chlamydia trachomatis*, first sexual intercourse, human papillomavirus, menarche, oral contraceptives

STRENGTHS AND LIMITATIONS OF THIS STUDY:

- A large HPV vaccination trial cohort with standardized clinical and laboratory procedures
- The repeated self-reported study questionnaires were less subject to recall bias
- Use of the overall cervical atypia end-point might dilute the effects

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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, squamous intraepithelial lesions (SIL) 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], life-time number of sexual partners,[8], age at the first sexual intercourse (FSI),[9], parity,[10] and use of oral contraceptives (OC),[11].

Both early age at the FSI and early age at the start of OC use are associated with an increased risk of SIL and CIN,[9, 12-14]. Furthermore, short lag between menarche and the 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 the oncogenic HPV types are established more readily in the immature cervix,[13, 15]. However, whether or not early start of OC use has an independent role here is open. The interplay of time interval between age at the start of OC use or age at the 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 the short interval between menarche and age at the start of OC use or age at the FSI.

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MATERIALS AND METHODS

Study Sample

The study population consists of women enrolled in the control arm of a double-blinded, multi-national randomized controlled PATRICIA trial (registration number NCT00122681) whose primary aim was to evaluate the vaccine efficacy (VE) of HPV16/18 vaccine against CIN2+,[16, 17]. Full descriptions 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 (2,409 received at least one dose of HPV16/18 vaccine) and 2,399 women (received at least one dose of hepatitis A-virus (HAV) vaccine). The criteria of having no more than 6 lifetime sexual partners was not applied in Finland, so all women interested and willing to participate the study were included,[18]. In Finland, the trial was approved by the Finnish national ethics committee (TUKIJA 1174/04) in 2004. 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 year-old when exiting the trial were sent a questionnaire on living conditions, life-habits and sexual health. All 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

The questionnaires collected information in addition to living-conditions and life-habits about the history of OC-use, other contraceptives use, smoking, menarche and sexual habits. The end-of study

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questionnaire was most complete as for start of sexual habits, and was used in the analysis. The age at the start of OC use, menarche and age at the FSI were the independent variables in this study. An interval of less than 3 years and more than or equal to 3 years was calculated between menarche and age at the start of OC use as well as between menarche and FSI. Data on smoking ('never-smokers', 'past smokers' and 'present smokers'), life-time number of sexual partners ('none', '1', '2-4', '5-9' and 'more than 10'), condom use ('non-user', 'user' and 'do not know') and sexually transmitted infections (HPV16/18 and *C. trachomatis*) were used as co-variables.

Laboratory analysis and endpoints

In the PATRICIA trial, semi-annual cervical cytological and DNA samples were obtained in conjunction of 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 cervical intraepithelial lesions (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 more than 3 years lag between menarche and FSI, 3) cervical atypia with shorter than 3 years lag between

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menarche and OC use and 4) cervical atypia with equal or more 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 a HPV vaccination trial in a questionnaire sent to house-holds (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 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.

Statistical analysis

The outcome variables were analysed in the univariate and multivariable logistic regression models along with the independent variables and above listed co-variates. The risks are reported as the odds ratios (OR) with95% confidence interval (CI). The statistical analysis was performed using Stata version 14.0 (Stata Corp LP, Statistical Software: Release 14. College Station, TX, USA).

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RESULTS

Baseline characteristics of our study cohort attending semiannual follow-up visits for four years are materially homogeneous with little variation (Table 1).

Table 1. Characteristics of young adult women (N=913) who attended eight semi-annual follow-up visits and a subgroup of these women (n=197) who developed cervical atypia during the four years of follow-up by the average age of 23 years

Characteristics	Attendees		Women with Atypia	
	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 first intercourse				
12-16	602	65.9	129	65.5
17-22	273	29.9	60	30.5
Missing	38	4.2	8	4.0
No. of sexual partners				
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
Oral contraceptive 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
HPV16				
Negative	711	77.9	145	73.6
Positive	201	22.0	52	26.4
Missing	1	0.1	0	0
HPV18				
Negative	792	86.8	165	83.8

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Positive	120	13.1	32	16.2
Missing	1	0.1	0	0
Chlamydia				
Negative	811	88.8	175	88.8
Positive	102	11.2	22	11.2
Missing	0	0	0	0

*LNSP= life-time number of sexual partners
#HPV16, HPV18 and *C.trachomatis* infections were recorded semi-annually for 4 years.

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.

By the end of the follow-up period, 156 (17.1%) of 913 women had ASCUS, 189 (20.7%) of 913 women had LSIL, 5 (0.6%) of 913 had HSIL, 40 (4.4%) of 913 had CIN1, 22 (2.41%) of 913 had CIN2 and 8 (0.9%) of 913 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 of 197, 28.0%) had had more than 10 sexual partners. Half of the women with or without cervical atypia (49.2%) and (45.4%) did not regularly use condoms. Most of the women (179 of 197, 90.9%) with cervical atypia had used oral contraceptives. Age at the start of OC use for a majority of these women (104 out of 197, 52.8%) was between 12 and 16 years (Table 1).

During the four year follow-up 201 (22%) of all women tested positive for HPV16 and 120 (13.1%) tested positive for HPV18 (Table 1). One third of women with either HPV16 or HPV18, or both were diagnosed with cervical atypia during the follow-up. The number of women testing positive

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for *C. trachomatis* was 102 (11.2%), and the number of *C. trachomatis* positive women with cervical atypia was 22 (11.2%) (Table1).

We categorized 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 the FSI using a stratification of <3 years and ≥3 years (Table 2).

Table 2. Distribution of cervical atypia risk factors by interval between menarche and age at the start of oral contraceptive (OC) use or between menarche and age at the first sexual intercourse (FSI) in young adult women followed up for 4 years.

Category	Interval between menarche and age at start of oral contraceptive use		Interval between menarche and age at first sexual intercourse	
	Interval <3 yrs. (N=192)	Interval ≥3 yrs. (N=675)	Interval <3 yrs. (N=302)	Interval ≥3 yrs. (N=566)
	n/Mean (%/SD)	n/Mean (%/SD)	n/Mean (%/SD)	n/Mean (%/SD)
C. 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 sexual debut	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)
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)

*LNSP=life-time number of sexual partners

The mean ages at menarche, at the 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 HPV16 positive

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than women in the ≥3 years interval categories (Table 2). The percentages of women with multiple (>5) life-time sexual partners were also higher in the short interval categories (Table 2).

In the univariate analysis, the risk of cervical atypia was evaluated separately in the short and long interval categories (Table 3).

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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+) stratified by the interval between menarche (M) and age at the 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			
	M to 1st intercourse <3 y SIL/CIN1+		M to 1st intercourse ≥3 y SIL/CIN1+		M to start of OCs <3 y SIL/CIN1+		M to start of OCs ≥3 y 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
Lag between 1st intercourse and menarche								
<3 yrs.	54/302	NA	NA	NA	31/192	NA	23/110	0.9 (0.5-1.4)
≥3 yrs	NA	NA	132/566	NA	NA	NA	132/565	1
Lag between start of OCs and menarche								
<3 yrs.	31/192	0.7 (0.4-1.3)	NA	NA	31/192	NA	NA	NA
≥3 yrs	23/110	1	132/565	NA	NA	NA	155/675	NA

*LNSP=lifetime number of sexual partner, high= 5 or more

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Cervical atypia risk estimates associated with HPV16/18 were increased (OR 1.8, CI: 1.1-2.7 and OR 1.4, CI: 1.0-2.1) in the longer (≥3 years) interval categories. On the contrary, the cervical atypia risk associated with *C. trachomatis* was increased (OR 1.8, CI 1.0-3.6; OR 2.2, CI: 1.0-5.1) 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). The risk of cervical atypia associated with the short interval between menarche and age at the start of OC use was somewhat decreased (OR 0.7, CI: 0.4-1.3) when also the interval between menarche and age at the FSI was short (Table 3). Also, there was no risk of atypia associated with the long interval between menarche and the start of OC use (Table 3). The risk remained the same even when the interval was entered as a continuous variable into the model.

In a multivariable analysis, including all the above-mentioned variables, the corresponding OR did not materially differ from that of univariate analysis but the upper 95% confidence limits approached 1 (OR 0.6, CI: 0.3-1.1) (data not shown). Stepwise exclusion of one variable at a time from the multivariable model was performed to check the interdependency of interval between menarche, age at the start of OC use, and age at the FSI in this context. Exclusion of any of the above-mentioned variables did not affect significance of the estimates (data not shown).

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DISCUSSION

We found that cervical atypia was not associated with the early start of sexual activity after menarche. The risk of cervical atypia associated with *C. trachomatis* was increased shortly after menarche, and the risk of cervical atypia associated with HPV16/18 infections more than three years after menarche.

Our large HPV vaccination trial derived population of young adult women, uniform ethnicity (97% Caucasian Finnish women), standardized clinical and laboratory procedures are noteworthy. In young Finnish women HIV infection has been and is extremely rare (www.thl.fi). Furthermore, over entire follow-up period the trial participants received regular sexual health counseling 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 age at the start of OC use with cervical atypia was now assessed for the first time.

Some limitations of our study are: Use of the overall cervical atypia end-point, which was necessary to retain 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 end-point questionnaire (at age 22) was, however, in line with the enrolment questionnaire (at age 18), e.g. for menarche. Moreover, questionnaire-based information on the sexual behavior is supposed to have adequate validity and reliability,[20, 21]. In addition, the participants were distributed free contraceptives during the trial period, which might have affected the proportions of OC and condom users in our study.

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The absence of HPV16/18 associated risk of cervical atypia in women with short lag between menarche and 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 age at the first sexual intercourse 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 with Ruiz et al. who first reported that short interval between menarche and age at the first sexual intercourse is a predictor of cervical cytological abnormalities and CIN,[9]. While our homogeneous study population had ample power to detect a three-fold increased risk (Appendix) their study population was heterogeneous. Furthermore, we found lack of association between short interval of menarche and two measures of start of sexual activity (age at the first intercourse and age at the start of OC use). However, the different observations on the interval between menarche and start of sexual activity, and 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 co-occurs 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 short lag between menarche and start of sexual activity emphasizes the need to identify, treat and follow-up adolescent females with *C. trachomatis*.

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In conclusion, while our study does not support the hypothesis that 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.

What is already known on this topic?

1. Both HPV infection and *Chlamydia trachomatis* are risk factors of cervical atypia.
2. The short interval between menarche and first sexual intercourse increases the risk of cytological abnormalities and CIN

What this study adds?

1. The short interval between menarche and first sexual intercourse or start of oral contraceptive use both increase the risk of cervical abnormalities in *C. trachomatis* positive women.
2. The cervical atypia risk is first associated with *C. trachomatis* later with HPV16/18

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Footnotes

Contributors: IA developed the research protocol, analyzed the data and prepared the manuscript. TE: data acquisition and data interpretation. TL: contributed to 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: data acquisition, contributed to the development of research plan, contributed to the analysis plan, commented on the draft of the paper and helped all the way in the revision of the paper.

Conflicts of interest: ML and DA have grants from Merck&Co. Inc. and GSK for HPV vaccination trials through their employers (University of Tampere, ML; Family Federation Finland, DA)

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Ethics approval: Finnish national ethics committee (TUKIJA 1174/04)

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Patient consent for publication: Not required

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Appendix. Power to find an association (p=0.05) for short interval between menarche and age at the start of sexual activity, and cervical atypia assuming up to 20% cumulative atypia incidence* and variable strength (odds ratio, OR) of the association in 900 women.

OR	**5%**	**10%**	**15%**	**20%**
0.5	0.22	0.49	0.72	0.87
2.0	0.64	0.90	0.98	1.00
4.0	1.00	1.00	1.00	1.00

*prevalence at the end of follow up

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Reported on page #
	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract -**longitudinal study**	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found- **We investigated whether the risk of cervical atypia is associated with a short interval between age at the first sexual intercourse (FSI) or age at the start of oral contraceptive (OC) use, and menarche. We found that a short interval between menarche and age at the start of oral contraceptive use do not increase the risk of cervical atypia.**	
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported- **Early age at the first sexual intercourse and early age at the start of oral contraceptive (OC) use are associated with an increased risk of SIL and CIN. The interplay of time interval between age at the start of OC use or age at the FSI, and menarche in cervical carcinogenesis has not been studied.**	1
Objectives	3	State specific objectives, including any prespecified hypotheses- **To investigate whether the risk of cervical atypia is associated with the short interval between menarche and age at the start of sexual activity.**	1
Methods			
Study design	4	Present key elements of study design early in the paper: **Longitudinal study including women (approx. 22 years) from the control arm (Hepatitis A vaccinated) of PATRICIA trial.**	2
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection-**The study was conducted in Finland. The study population consisted of women enrolled in the control arm of a double-blinded, multi-national randomized controlled PATRICIA trial. The women who had answered behavioral questionnaire after exiting from the trial in 2010 and were non-HPV vaccinated were enrolled in the present study.**	2
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up-**The participants had semi-annual follow-up when they were in the trial.**	3
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed- **NA**	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable-**Outcome cervical atypia**. **Exposure- menarche, age at**	3

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		first sexual intercourse and age at first use of oral contraceptive	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group-**Sexual and behavioural data were obtained from the questionnaire.**	2
Bias	9	Describe any efforts to address potential sources of bias- **The standardized clinical and laboratory procedures used in trial might have reduced the bias earlier in the study. Sexual health counselling of the participants during the follow-up also reduced the chances of bias.**	10
Study size	10	Explain how the study size was arrived at –**described above**	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why- **Quantitative variables such as age and number of sexual partners were categorised into different categories.**	2,3
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **NA**	
		(*b*) Describe any methods used to examine subgroups and interactions **NA**	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed- **potentially eligible 2098 women, after examining for eligibility 999, confirmed eligible and included in the study 914 women.**	2, 3
		(b) Give reasons for non-participation at each stage **Initially those who answered the questionnaire were all eligible. Later those who were HPV vaccinated were excluded. Finally the baseline cases were excluded.**	2
		(c) Consider use of a flow diagram **Flow diagram is presented in our earlier paper which is cited in this paper.**	2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders- **16-17 years old female from Finland were enrolled in the PATRICIA trial who were approximately 22 years old when exiting the trial.**	2
		(b) Indicate number of participants with missing data for each variable of interest-**Age at menarche 8, Age at first intercourse 38, Age at start of OC use 38. Shown in table 1**	5
		(c) Summarise follow-up time (eg, average and total amount) **4 years**	2
Outcome data	15*	Report numbers of outcome events or summary measures over time	**198 cervical atypia cases**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included-**0.9 (0.6-1.5) and 0.7 (0.4-1.3)**	8
		(*b*) Report category boundaries when continuous variables were	

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		categorized –**inclusive class interval**	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **NA**	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **NA**	
Discussion			
Key results	18	Summarise key results with reference to study objectives.**Increased risk of cervical atypia was found associated with *C. trachomatis* infection in women with short interval between menarche and start of sexual activity. Increased risk of cervical atypia was found associated with HPV16/18 infections in women with longer interval between menarche and start of sexual activity. Early start of OC-use may not increase the risk of atypia**	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias- **The questionnaire data were self-reported at the age of 22 years which is subject to recall bias.**	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence – **while our study does not support the hypothesis that short interval between menarche and age at the start of sexual activity increases the risk of cervical atypia early age of acquiring *C. trachomatis* infections is setting the stage for cervical carcinogenesis.**	11
Generalisability	21	Discuss the generalisability (external validity) of the study results- **This study is generalizable to the similar population of young women with more than 6 life time number of sexual partners as well as to those women who had an easy access to the contraceptive methods**.	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based-: **Matti Lehtinen and Dan Apter have grants from Merck & Co. Inc. and GSK for HPV vaccination trials through their employers (University of Tampere, ML; Family Federation Finland, DA)**	

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely

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available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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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 age at the first sexual intercourse (FSI) or age at the start of oral contraceptive (OC) use, and menarche.

Design A population-based cohort study.

Setting 16-17 year-old Finnish women 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 semi-annual clinical follow-up visits for four years. Altogether 913 women had normal baseline cervical cytology and answered behavioural questionnaires at enrolment and end of the follow-up.

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

Results The mean ages at menarche, at FSI and at the start of OC use were 12.4, 16.0 and 16.4. *C. trachomatis* infection was associated with an increased risk of cervical atypia in women with a short (<3 years) interval between menarche and the FSI/start of OC use (odds ratio, OR 1.8, 95% confidence interval [CI]: 1.0-3.6, and OR 2.2 95% CI: 1.0-5.1) whereas HPV16/18 infection was associated with increased atypia risk estimates in women with a longer (≥3 years) interval (OR 1.8, 95% CI: 1.1-2.7, and OR 1.4. 95% CI: 1.0-2.1). In women with a short interval between menarche and FSI, early age at start of OC use was not associated with an increased risk of cervical atypia neither in univariate (OR 0.7) nor in multivariable analyses.

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

Key words cervical neoplasia, *Chlamydia trachomatis*, first sexual intercourse, human papillomavirus, menarche, oral contraceptives

STRENGTHS AND LIMITATIONS OF THIS STUDY:

- A large HPV vaccination trial cohort with standardized clinical and laboratory procedures
- The repeated self-reported study questionnaires were comprehensive and less subject to recall bias
- Use of the overall cervical atypia end-point increases study power but might have diluted the effects

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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, squamous intraepithelial lesions (SIL) 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], life-time number of sexual partners,[8], age at the first sexual intercourse (FSI),[9], parity,[10] and use of oral contraceptives (OC),[11].

Both early age at the FSI and early age at the start of OC use are associated with an increased risk of SIL and CIN,[9, 12-14]. Furthermore, short lag between menarche and the 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 the oncogenic HPV types are established more readily in the immature cervix,[13, 15]. However, whether or not early start of OC use has an independent role here is open. The interplay of time interval between age at the start of OC use or age at the 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 the short interval between menarche and age at the start of OC use or age at the FSI.

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MATERIALS AND METHODS

Study Sample

The study population consists of women enrolled in the control arm of a double-blinded, multi-national randomized controlled PATRICIA trial (registration number NCT00122681) whose primary aim was to evaluate the vaccine efficacy (VE) of HPV16/18 vaccine against CIN2+,[16, 17]. Full descriptions 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 (2,409 received at least one dose of HPV16/18 vaccine) and 2,399 women (received at least one dose of hepatitis A-virus (HAV) vaccine). The criteria of having no more than 6 lifetime sexual partners was not applied in Finland, so all women interested and willing to participate the study were included,[18].In Finland, the trial was approved by the Finnish national ethics committee (TUKIJA 1174/04) in 2004.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 year-old when exiting the trial were sent a questionnaire on living conditions, life-habits and sexual health. All 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

The questionnaires collected information in addition to living-conditions and life-habits about the history of OC-use, other contraceptives use, smoking, menarche and sexual habits. The end-of study

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questionnaire was most complete as for start of sexual habits, and was used in the analysis. The age at the start of OC use, menarche and age at the FSI were the independent variables in this study. An interval of less than 3 years and more than or equal to 3 years was calculated between menarche and age at the start of OC use as well as between menarche and FSI. Data on smoking ('never-smokers', 'past smokers' and 'present smokers'), life-time number of sexual partners ('none', '1', '2-4', '5-9' and 'more than 10'), condom use ('non-user', 'user' and 'do not know') and sexually transmitted infections (HPV16/18 and *C. trachomatis*) were used as co-variables as they are an important factors in cervical carcinogenesis. These co-variables were used in both univariate and multivariable models to evaluate if the short intervals between menarche and FSI or age at the start of OC are truly associated with or modify the risk of cervical atypia.

Laboratory analysis and endpoints

In the PATRICIA trial, semi-annual cervical cytological and DNA samples were obtained in conjunction of 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 cervical intraepithelial lesions (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.

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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 more 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 more 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 a HPV vaccination trial in a questionnaire sent to house-holds (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 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.

Statistical analysis

The outcome variables were analysed in the univariate and multivariable logistic regression models along with the independent variables and above listed co-variates. The risks are reported as the odds ratios (OR) with 95% confidence interval (CI). The statistical analysis was performed using Stata version 14.0 (Stata Corp LP, Statistical Software: Release 14. College Station, TX, USA).

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RESULTS

Baseline characteristics of our study cohort attending semiannual follow-up visits for four years are materially homogeneous with little variation (Table 1).

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

Characteristics	Attendees		Women with Atypia	
	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 first intercourse				
12-16	602	65.9	129	65.5
17-22	273	29.9	60	30.5
Missing	38	4.2	8	4.0
No. of sexual partners				
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
Oral contraceptive 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
HPV16				
Negative	711	77.9	145	73.6
Positive	201	22.0	52	26.4
HPV18				
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

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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, 156 (17.1%) of 913 women had ASCUS, 189 (20.7%) of 913 women had LSIL, 5 (0.6%) of 913 had HSIL, 40 (4.4%) of 913 had CIN1, 22 (2.41%) of 913 had CIN2 and 8 (0.9%) of 913 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 of 197, 28.0%) had had more than 10 sexual partners. Half of the women with or without cervical atypia (49.2%) and (45.4%) did not regularly use condoms. Most of the women (179 of 197, 90.9%) with cervical atypia had used oral contraceptives. Age at the start of OC use for a majority of these women (104 out of 197, 52.8%) was between 12 and 16 years (Table 1).

During the four year follow-up 201 (22%) of all women tested positive for HPV16 and 120 (13.1%) tested positive for HPV18 (Table 1). One third of women with either HPV16 or HPV18, or both were diagnosed with cervical atypia during the follow-up. The number of women testing 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).

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We categorized 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 the FSI using a stratification of <3 years and ≥3 years (Table 2).

Table 2. Distribution of cervical atypia risk factors by interval between menarche and age at the start of oral contraceptive (OC) use or age at the first sexual intercourse (FSI) in young adult women followed up for 4 years.

Category	Interval between menarche and age at the start of OC-use		Interval between menarche and the FSI	
	Interval <3 yrs. (N=192)	Interval ≥3 yrs. (N=675)	Interval <3 yrs. (N=302)	Interval ≥3 yrs. (N=566)
	n/Mean (%/SD)	n/Mean (%/SD)	n/Mean (%/SD)	n/Mean (%/SD)
C. 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 sexual debut	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)
Life-time 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)

The mean ages at menarche, at the 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 HPV16 positive than women in the ≥3 years interval categories (Table 2). The percentages of women with multiple (>5) life-time sexual partners 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).

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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 the 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 SIL/CIN1+		Menarche to FSI ≥3 y SIL/CIN1+		Menarche to start of OCs <3 y SIL/CIN1+		Menarche to start of OCs ≥3 y 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 partner, high= 5 or more

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Cervical atypia risk estimates associated with HPV16/18 were increased (OR 1.8, CI: 1.1-2.7 and OR 1.4, CI: 1.0-2.1) in the longer (≥3 years) interval categories. On the contrary, the cervical atypia risk associated with *C. trachomatis* was increased (OR 1.8, CI 1.0-3.6; OR 2.2, CI: 1.0-5.1) 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, CI: 0.4-1.3) when the interval between menarche and age at the FSI was short (Table 4).

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 the 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 ≥3 y**		
	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	

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).

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

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DISCUSSION

We found that cervical atypia was not associated with the 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 HPV16/18 infections more than three years after start of sexual activity following menarche.

Our large HPV vaccination trial derived population of young adult women, uniform ethnicity (97% Caucasian Finnish women), standardized clinical and laboratory procedures are noteworthy. In young Finnish women HIV infection has been and is extremely rare (www.thl.fi). Furthermore, over entire follow-up period the trial participants received regular sexual health counseling 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 age at the start of OC use with cervical atypia was now assessed for the first time.

Some limitations of our study are: Use of the overall cervical atypia end-point, which was necessary to retain 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 end-point questionnaire (at age 22) was, however, in line with the enrolment questionnaire (at age 18), e.g. for menarche. Moreover, questionnaire-based information on the sexual behavior is supposed to have adequate validity and reliability,[20, 21]. It gave 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. The

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participants were distributed free contraceptives during the trial period, which might have increased the proportions of OC and condom users in our study.

The absence of HPV16/18 associated risk of cervical atypia in women with short lag between menarche and 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 age at the first sexual intercourse 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 with Ruiz et al. who first reported that short interval between menarche and age at the first sexual intercourse is a predictor of cervical cytological abnormalities and CIN,[9]. While our homogeneous study population had ample power to detect a three-fold increased risk (Appendix) their study population was heterogeneous, and had but baseline sexual risk-taking behavior questionnaire data, which could not elaborate (possible changes in) the risk-taking behavior during the follow-up. Furthermore, we found lack of association between short interval of menarche and two different measures of start of sexual activity (age at the first intercourse and age at the start of OC use). However, these different observations on the interval between menarche and start of sexual activity, and 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 co-occurs with HPV infection the risk of high-grade cervical neoplasia associated with the joint infection is very

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high,[23]. Our results on the increased risk of *C. trachomatis* infection with cervical atypia especially in women with short lag between menarche and start of sexual activity emphasizes the need to identify, treat and follow-up adolescent females with *C. trachomatis.*

In conclusion, while our study does not support the hypothesis that 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.

What is already known on this topic?

1. Both HPV infection and *Chlamydia trachomatis* are risk factors of cervical atypia.
2. The short interval between menarche and first sexual intercourse may increase the risk of cytological abnormalities and CIN

What this study adds?

1. The cervical atypia risk is first associated with *C. trachomatis* later with HPV16/18 infection.
2. The risk of cervical atypia in *C. trachomatis* positive women is seen especially in women with short lag between menarche and the first sexual intercourse or the start of oral contraceptive use.

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Footnotes

Contributors: IA developed the research protocol, analyzed the data and prepared the manuscript. TE: data acquisition and data interpretation. TL: contributed to 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: data acquisition, contributed to the development of research plan, contributed to the analysis plan, commented on the draft of the paper and helped all the way in the revision of the paper.

Conflicts of interest: ML and DA have grants from Merck & Co. Inc. and GSK for HPV vaccination trials through their employers (Tampere University, ML; Family Federation Finland, DA)

Financial Support: Academy of Finland

Ethics approval: Finnish national ethics committee (TUKIJA 1174/04)

Data Sharing Statement: No additional data available

Patient consent for publication: Not required

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Appendix. Power to find an association (p=0.05) for short interval between menarche and age at the start of sexual activity, and cervical atypia assuming up to 20% cumulative atypia incidence* and variable strength (odds ratio, OR) of the association in 900 women.

OR	**5%**	**10%**	**15%**	**20%**
0.5	0.22	0.49	0.72	0.87
2.0	0.64	0.90	0.98	1.00
4.0	1.00	1.00	1.00	1.00

*prevalence at the end of follow up

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Reported on page #
	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract -**longitudinal study**	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found- **We investigated whether the risk of cervical atypia is associated with a short interval between age at the first sexual intercourse (FSI) or age at the start of oral contraceptive (OC) use, and menarche. We found that a short interval between menarche and age at the start of oral contraceptive use do not increase the risk of cervical atypia.**	
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported- **Early age at the first sexual intercourse and early age at the start of oral contraceptive (OC) use are associated with an increased risk of SIL and CIN. The interplay of time interval between age at the start of OC use or age at the FSI, and menarche in cervical carcinogenesis has not been studied.**	1
Objectives	3	State specific objectives, including any prespecified hypotheses- **To investigate whether the risk of cervical atypia is associated with the short interval between menarche and age at the start of sexual activity.**	1
Methods			
Study design	4	Present key elements of study design early in the paper: **Longitudinal study including women (approx. 22 years) from the control arm (Hepatitis A vaccinated) of PATRICIA trial.**	2
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection-**The study was conducted in Finland. The study population consisted of women enrolled in the control arm of a double-blinded, multi-national randomized controlled PATRICIA trial. The women who had answered behavioral questionnaire after exiting from the trial in 2010 and were non-HPV vaccinated were enrolled in the present study.**	2
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up-**The participants had semi-annual follow-up when they were in the trial.**	3
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed- **NA**	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable-**Outcome cervical atypia**. **Exposure- menarche, age at**	3

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		first sexual intercourse and age at first use of oral contraceptive	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group-**Sexual and behavioural data were obtained from the questionnaire.**	2
Bias	9	Describe any efforts to address potential sources of bias- **The standardized clinical and laboratory procedures used in trial might have reduced the bias earlier in the study. Sexual health counselling of the participants during the follow-up also reduced the chances of bias.**	10
Study size	10	Explain how the study size was arrived at –**described above**	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why- **Quantitative variables such as age and number of sexual partners were categorised into different categories.**	2,3
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **NA**	
		(*b*) Describe any methods used to examine subgroups and interactions **NA**	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed- **potentially eligible 2098 women, after examining for eligibility 999, confirmed eligible and included in the study 914 women.**	2, 3
		(b) Give reasons for non-participation at each stage **Initially those who answered the questionnaire were all eligible. Later those who were HPV vaccinated were excluded. Finally the baseline cases were excluded.**	2
		(c) Consider use of a flow diagram **Flow diagram is presented in our earlier paper which is cited in this paper.**	2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders- **16-17 years old female from Finland were enrolled in the PATRICIA trial who were approximately 22 years old when exiting the trial.**	2
		(b) Indicate number of participants with missing data for each variable of interest-**Age at menarche 8, Age at first intercourse 38, Age at start of OC use 38. Shown in table 1**	5
		(c) Summarise follow-up time (eg, average and total amount) **4 years**	2
Outcome data	15*	Report numbers of outcome events or summary measures over time	**198 cervical atypia cases**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included-**0.9 (0.6-1.5) and 0.7 (0.4-1.3)**	8
		(*b*) Report category boundaries when continuous variables were	

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		categorized –**inclusive class interval**	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **NA**	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **NA**	
Discussion			
Key results	18	Summarise key results with reference to study objectives.**Increased risk of cervical atypia was found associated with *C. trachomatis* infection in women with short interval between menarche and start of sexual activity. Increased risk of cervical atypia was found associated with HPV16/18 infections in women with longer interval between menarche and start of sexual activity. Early start of OC-use may not increase the risk of atypia**	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias- **The questionnaire data were self-reported at the age of 22 years which is subject to recall bias.**	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence – **while our study does not support the hypothesis that short interval between menarche and age at the start of sexual activity increases the risk of cervical atypia early age of acquiring *C. trachomatis* infections is setting the stage for cervical carcinogenesis.**	11
Generalisability	21	Discuss the generalisability (external validity) of the study results- **This study is generalizable to the similar population of young women with more than 6 life time number of sexual partners as well as to those women who had an easy access to the contraceptive methods**.	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based-: **Matti Lehtinen and Dan Apter have grants from Merck & Co. Inc. and GSK for HPV vaccination trials through their employers (University of Tampere, ML; Family Federation Finland, DA)**	

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely

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available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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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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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 16-17 year-old Finnish women 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 four years. Altogether, 913 women had normal baseline cervical cytology and answered behavioural questionnaires at enrolment and end of the follow-up.

Main outcome measure Odds ratios with 95% confidence intervals 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. *C. 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 (odds ratio, OR 1.8, 95% confidence interval [CI]: 1.0-3.6, and OR 2.2 95% CI: 1.0-5.1). Whereas HPV16/18 infection was associated with increased atypia risk estimates in women with a longer (≥3 years) interval (OR 1.8, 95% CI: 1.1-2.7, and OR 1.4. 95% CI: 1.0-2.1). 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.

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

Key words cervical neoplasia, *Chlamydia trachomatis*, first sexual intercourse, human papillomavirus, menarche, oral contraceptives

STRENGTHS AND LIMITATIONS OF THIS STUDY:

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

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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 (SIL) 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], life-time number of sexual partners,[8], age at first sexual intercourse (FSI),[9], parity,[10] and the use of oral contraceptives (OC),[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 age at 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 age at FSI.

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MATERIALS AND METHODS

Study Sample

The study population consists of women enrolled in the control arm of a double-blinded, multi-national randomized control PATRICIA trial (registration number NCT00122681) whose primary aim was to evaluate the vaccine efficacy (VE) of the HPV16/18 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 (2,409 received at least one dose of HPV16/18 vaccine) and 2,399 women (received at least one dose of hepatitis A-virus (HAV) vaccine). The criteria of having no more than 6 lifetime sexual partners was not applied in Finland, so all women interested and willing to participate in the study were included,[18]. In Finland, the trial was approved by the Finnish national ethics committee (TUKIJA 1174/04) in 2004. 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, life-style-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 life-habits, the questionnaires collected information about history of OC use, use of other contraceptives, smoking, menarche and

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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 less than 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'), life-time number of sexual partners ('none', '1', '2-4', '5-9' and 'more than 10'), condom use ('non-user', 'user' and 'do not know') and sexually transmitted infections (HPV16/18 and *C. trachomatis*) were used as co-variables, as they are an important factors in cervical carcinogenesis. These co-variables 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 cervical intraepithelial lesions (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.

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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 house-holds (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.

Statistical analysis

The outcome variables were analysed in the univariate and multivariable logistic regression models along with the independent variables and above listed co-variates. The risks are reported as the odds ratios (OR) with 95% confidence interval (CI). The statistical analysis was performed using Stata version 14.0 (Stata Corp LP, Statistical Software: Release 14. College Station, TX, USA).

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RESULTS

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

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 four years of follow-up.

Characteristics	Attendees		Women with Atypia	
	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 first intercourse				
12-16	602	65.9	129	65.5
17-22	273	29.9	60	30.5
Missing	38	4.2	8	4.0
No. of sexual partners				
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
Oral contraceptive 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
HPV16				
Negative	711	77.9	145	73.6
Positive	201	22.0	52	26.4
HPV18				
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

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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 of 197, 28.0%) had had more than 10 sexual partners. 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 out of 197, 90.9%) with cervical atypia had used oral contraceptives. Age at the start of OC use for the majority of these women (104 out of 197, 52.8%) was between 12 and 16 years (Table 1).

During the four year follow-up, 201 (22%) of all women tested positive for HPV16 and 120 (13.1%) tested positive for HPV18 (Table 1). 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%) (Table 1).

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We categorized 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).

Table 2. Distribution of cervical atypia risk factors by interval between menarche and age at the start of oral contraceptive (OC) use or age at the first sexual intercourse (FSI) in young adult women followed up for 4 years.

Category	Interval between menarche and age at the start of OC-use		Interval between menarche and the FSI	
	Interval <3 yrs. (N=192)	Interval ≥3 yrs. (N=675)	Interval <3 yrs. (N=302)	Interval ≥3 yrs. (N=566)
	n/Mean (%/SD)	n/Mean (%/SD)	n/Mean (%/SD)	n/Mean (%/SD)
C. 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 sexual debut	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)
Life-time 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)

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 HPV16 positive than women in the ≥3 years interval categories (Table 2). The percentages of women with multiple (>5) life-time sexual partners 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).

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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 SIL/CIN1+		Menarche to FSI ≥3 y SIL/CIN1+		Menarche to start of OCs <3 y SIL/CIN1+		Menarche to start of OCs ≥3 y 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 partner, high= 5 or more

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Cervical atypia risk estimates associated with HPV16/18 were increased (OR 1.8, CI: 1.1-2.7 and OR 1.4, CI: 1.0-2.1) in the longer (≥3 years) interval categories. On the contrary, the cervical atypia risk associated with *C. trachomatis* was increased (OR 1.8, CI 1.0-3.6; OR 2.2, CI: 1.0-5.1) 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, CI: 0.4-1.3) when the interval between menarche and age at the FSI was short (Table 4).

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 ≥3 y**		
	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	

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).

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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 above-mentioned variables did not affect significance of the estimates (data not shown).

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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 HPV16/18 infections more than three 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 standardized 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 counseling 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 end-point, 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 end-point 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 behavior 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

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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 HPV16/18 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 first sexual intercourse 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 first sexual intercourse is a predictor of cervical cytological abnormalities and CIN,[9]. While our homogeneous study population had ampler power to detect a three-fold increased risk (Appendix) their study population was heterogeneous, and had only baseline sexual risk-taking behavior questionnaire data, which could not elaborate (possible changes in) the risk-taking behavior 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 first intercourse 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 co-occurs with HPV infection the risk of high-grade cervical neoplasia associated with the joint infection is very

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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 emphasizes 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.

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Footnotes

Contributors: IA developed the research protocol, analyzed the data and prepared the manuscript. TE: 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: data acquisition, contributed in the development of research plan, contributed in the analysis plan, commented on the draft of the paper and revision of the paper.

Conflicts of interest: ML and DA have grants from Merck & Co. Inc. and GSK for HPV vaccination trials through their employers (Tampere University, ML; Family Federation Finland, DA)

Financial Support: Academy of Finland

Ethics approval: Finnish national ethics committee (TUKIJA 1174/04)

Data Sharing Statement: No additional data available

Patient consent for publication: Not required

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Appendix. Power to find an association (p=0.05) for short interval between menarche and age at the start of sexual activity, and cervical atypia assuming up to 20% cumulative atypia incidence* and variable strength (odds ratio, OR) of the association in 900 women.

OR	**5%**	**10%**	**15%**	**20%**
0.5	0.22	0.49	0.72	0.87
2.0	0.64	0.90	0.98	1.00
4.0	1.00	1.00	1.00	1.00

*prevalence at the end of follow up

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Reported on page #
	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract -**longitudinal study**	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found- **We investigated whether the risk of cervical atypia is associated with a short interval between age at the first sexual intercourse (FSI) or age at the start of oral contraceptive (OC) use, and menarche. We found that a short interval between menarche and age at the start of oral contraceptive use do not increase the risk of cervical atypia.**	
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported- **Early age at the first sexual intercourse and early age at the start of oral contraceptive (OC) use are associated with an increased risk of SIL and CIN. The interplay of time interval between age at the start of OC use or age at the FSI, and menarche in cervical carcinogenesis has not been studied.**	1
Objectives	3	State specific objectives, including any prespecified hypotheses- **To investigate whether the risk of cervical atypia is associated with the short interval between menarche and age at the start of sexual activity.**	1
Methods			
Study design	4	Present key elements of study design early in the paper: **Longitudinal study including women (approx. 22 years) from the control arm (Hepatitis A vaccinated) of PATRICIA trial.**	2
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection-**The study was conducted in Finland. The study population consisted of women enrolled in the control arm of a double-blinded, multi-national randomized controlled PATRICIA trial. The women who had answered behavioral questionnaire after exiting from the trial in 2010 and were non-HPV vaccinated were enrolled in the present study.**	2
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up-**The participants had semi-annual follow-up when they were in the trial.**	3
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed- **NA**	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable-**Outcome cervical atypia**. **Exposure- menarche, age at**	3

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		first sexual intercourse and age at first use of oral contraceptive	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group-**Sexual and behavioural data were obtained from the questionnaire.**	2
Bias	9	Describe any efforts to address potential sources of bias- **The standardized clinical and laboratory procedures used in trial might have reduced the bias earlier in the study. Sexual health counselling of the participants during the follow-up also reduced the chances of bias.**	10
Study size	10	Explain how the study size was arrived at –**described above**	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why- **Quantitative variables such as age and number of sexual partners were categorised into different categories.**	2,3
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **NA**	
		(*b*) Describe any methods used to examine subgroups and interactions **NA**	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed- **potentially eligible 2098 women, after examining for eligibility 999, confirmed eligible and included in the study 914 women.**	2, 3
		(b) Give reasons for non-participation at each stage **Initially those who answered the questionnaire were all eligible. Later those who were HPV vaccinated were excluded. Finally the baseline cases were excluded.**	2
		(c) Consider use of a flow diagram **Flow diagram is presented in our earlier paper which is cited in this paper.**	2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders- **16-17 years old female from Finland were enrolled in the PATRICIA trial who were approximately 22 years old when exiting the trial.**	2
		(b) Indicate number of participants with missing data for each variable of interest-**Age at menarche 8, Age at first intercourse 38, Age at start of OC use 38. Shown in table 1**	5
		(c) Summarise follow-up time (eg, average and total amount) **4 years**	2
Outcome data	15*	Report numbers of outcome events or summary measures over time	**198 cervical atypia cases**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included-**0.9 (0.6-1.5) and 0.7 (0.4-1.3)**	8
		(*b*) Report category boundaries when continuous variables were	

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		categorized –**inclusive class interval**	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **NA**	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **NA**	
Discussion			
Key results	18	Summarise key results with reference to study objectives.**Increased risk of cervical atypia was found associated with *C. trachomatis* infection in women with short interval between menarche and start of sexual activity. Increased risk of cervical atypia was found associated with HPV16/18 infections in women with longer interval between menarche and start of sexual activity. Early start of OC-use may not increase the risk of atypia**	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias- **The questionnaire data were self-reported at the age of 22 years which is subject to recall bias.**	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence – **while our study does not support the hypothesis that short interval between menarche and age at the start of sexual activity increases the risk of cervical atypia early age of acquiring *C. trachomatis* infections is setting the stage for cervical carcinogenesis.**	11
Generalisability	21	Discuss the generalisability (external validity) of the study results- **This study is generalizable to the similar population of young women with more than 6 life time number of sexual partners as well as to those women who had an easy access to the contraceptive methods**.	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based-: **Matti Lehtinen and Dan Apter have grants from Merck & Co. Inc. and GSK for HPV vaccination trials through their employers (University of Tampere, ML; Family Federation Finland, DA)**	

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely

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available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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