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European Journal of Cancer 40 (2004) 1058–1065

European Journal of Cancer

www.ejconline.com

Joint effects of different human papillomaviruses and *Chlamydia trachomatis* infections on risk of squamous cell carcinoma of the cervix uteri

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Received 18 November 2003; accepted 25 November 2003

Abstract

This case–control study based in Nordic serum banks evaluated the joint effects of infections with genital human papillomavirus (HPV) types, and *Chlamydia trachomatis* in the aetiology of cervical squamous cell carcinoma. Through a linkage with the cancer registries, 144 cases were identified and 420 controls matched to them. Exposure to past infections was defined by the presence of specific IgG antibodies. The odds ratio (OR) for the second-order interaction of HPV16, HPV6/11 and *C. trachomatis* was small (1.0) compared to the expected multiplicative OR, 57, and the additive OR, 11. The interactions were not materially different among HPV16 DNA-positive squamous cell carcinomas. When HPV16 was replaced with HPV18/33 in the analysis of second-order interactions with HPV6/11 and *C. trachomatis*, there was no evidence of interaction, the joint effect being close to the expected additive OR. Possible explanations for the observed antagonism include misclassification, selection bias or a true biological phenomenon with HPV6/11 and *C. trachomatis* exposures antagonizing the carcinogenic effects of HPV16.

Keywords: Cervix; Interaction; Neoplasms; Papillomaviruses; *Chlamydia trachomatis*

1. Introduction

Cancer of the uterine cervix is the second most common cancer among women in the developing countries and still a common cancer in the Northern Europe [1]. Infection with oncogenic human papillomavirus (HPV) types (notably types 16 and 18) is the major risk factor for invasive cervical cancer [2]. Longitudinal studies of invasive cervical cancer have confirmed the role of past infection with HPV16 and 18 as a risk factor [3–5]. Past infections with *Chlamydia trachomatis*, and HPV types 6 and 11, may be associated with an increased risk of squamous cell carcinoma (SCC) of the uterine cervix in longitudinal studies [6–9].

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doi:10.1016/j.ejca.2003.11.032

A case–control study based on Nordic serum banks in 1973 to 1994 suggested that the joint effects of HPV16 and HPV6/11, and HPV16 and *C. trachomatis*, were antagonistic, i.e. smaller than expected on the basis of the multiplicative effects [6,7]. The present study seeks to specify the results of the previous studies. It hopes to provide a clearer picture of the role of risk factors interacting with HPV16 in cervical carcinogenesis by focusing on HPV16 DNA-carrying cancers and a more accurate case ascertainment by histological reclassification of the registered diagnoses. It also investigates the possible role of confounding by a sexually transmitted infection, herpes simplex virus type 2 (HSV-2).

2. Materials and methods

2.1. Serum banks

The study cohort consisted of approximately 550 000 women who donated blood samples to population-based serum banks in Norway, Finland and Sweden (Table 1).

The JANUS project was initiated in Norway in 1973 to search in premorbid serum samples for biomarkers in cancer epidemiology [10]. Women were recruited into the project during routine health examinations. In 1991 the JANUS serum bank contained serum samples stored at −25 °C from about 145 000 women. The participation rate was 85% during 1974 to1978 and 75% during 1986–1991.

The Finnish Maternity Cohort started to collect blood samples during early pregnancy for screening purposes in Finland in 1983 [11]. About 98% of pregnant women have donated blood samples drawn at maternity clinics to the bank. In 1993 the bank contained 710 000 blood samples from 390 000 women, stored at −25 °C.

The Västerbotten Project was initiated in a northern Swedish county in 1986 [12]. Each year all residents aged 30, 40, 50 and 60 years are invited to participate in a health-promoting project, including the donation of biological samples. The participation rate is about 65%. In 1993 the bank contained samples from 15 000 women, stored at −80 °C.

2.2. Cancer registries

The Cancer Registry of Norway and the Finnish Cancer Registry are nationwide [13], and the regional cancer registry in Umeå covers the four northern-most counties in Sweden [14]. The registries are population-based, and achieve close to 100% coverage in reporting.

2.3. Identification of cases, confirmation of histology, and matching

A total of 223 cases (women with invasive cervical cancer as their first primary cancer) were identified in 1994 by linking the data files of the serum banks and the cancer registries using personal identification numbers. 37 cases were excluded: 29 could not be located, four had donated serum sample less than half a month before the diagnosis, and four were carcinoma-in-situ lesions.

The histological slides of 182 cases included in the earlier studies [4,6–8] and of four cases excluded from those studies (one leiomyosarcoma and three without reported histology) were retrieved for histological reclassification by two pathologists (V.A. and E.S.) [15]. Eight exclusions were made: one endometrial carcinoma, one non-located without reported histology, and six cases interpreted as precancerous with no clinical information about invasion. The histologically confirmed invasive cervical carcinomas were not excluded if the histological specimen for the review was missing or non-representative. Finally, there were 178 cases of invasive cervical carcinoma: 148 SCC (107 reconfirmed) and 30 of other histological types.

For each case, three cancer-free controls were randomly selected, matched for sex, age at the first serum sampling (±2 years), storage time of the first serum sample (±2 months), and area of residence (Finland, Northern Swedish and 11 Norwegian counties). If three controls

Table 1
Selected characteristics of the Nordic serum banks used as source for the cohort of nested case-control study on cervical cancer risk due to sexually transmitted infections

Serum bank	Geographic location	Period of serum sampling	No. of female donors	No. of		Age* at	
				Cases	Controls	Serum sampling	Diagnosis
Janus Serum Bank	Three counties in Norway[a]	1974–78	29 000	79	237	21–50	26–64
Janus Serum Bank	Several counties in Norway	1986–91	116 000	47	141	34–62	35–63
Västerbotten Project	A county in Northern Sweden[b]	1986–	15 000	4	12	39–60	40–61
Finnish Maternity Cohort	Finland	1983–	390 000	48	137	19–41	23–49
Total	Nordic	1974–	550 000	178	527	19–62	23–64

*Years.
[a] Counties of Finnmark, Oppland and Sogn og Fjordane.
[b] County of Västerbotten.

for a case could not be found, the matching criteria on age and storage time were widened. The serum samples of six controls for SCC cases could not be located.

The main focus of this study was SCC of the cervix uteri. The number of SCC cases and control samples sent for laboratory analyses were 148 and 438, respectively. Four case-control sets with incomplete case information and eight controls with missing information about the key agents, HPV types 6/11 and 16 and *C. trachomatis*, were excluded. The final numbers of cases and controls were 144 and 420, respectively.

2.4. Laboratory methods

2.4.1. Serology

IgG antibodies to oncogenic (16, 18 and 33) and non-oncogenic (6 and 11) HPV types were determined by the standard enzyme-linked immunosorbent assay using HPV capsids, monoclonal antihuman IgG and horseradish peroxidase-labelled goat antimouse IgG as conjugate [16]. The cut-offs for assigning seropositivity were the same as validated in previous studies [4]. IgG antibodies specific to *C. trachomatis* were determined by the microimmunofluorescence method [17] using serovar pools B-group (B-E-D), C-group (C-H-I-J) and an intermediate group (G-F-K) (Washington Research Foundation, Seattle, WA). Titers of 1:16 or more were considered positive for *C. trachomatis*. IgG antibodies to HSV-2 were determined by using a commercially available HSV-2 glycoprotein G-2 enzyme-linked immunosorbent assay (Biokit SA, Barcelona, Spain) according to the manufacturer's recommendations. Serum cotinine was measured by radioimmunoassay using a hapten-specific monoclonal antibody [18]. A serum cotinine of 20 ng/ml and above was considered to indicate active smoking. The laboratory analyses were performed with masked samples.

2.4.2. HPV DNA detection

Formalin-fixed, paraffin-embedded biopsy specimens from 133 cases were examined for HPV DNA by polymerase chain reaction (PCR) using the L1 consensus primers GP5+/6+ [19]. Sections from an empty paraffin block were cut between each case and used as a contamination control for each PCR. HPV DNA-positive cases were typed with E6- and E7-derived, type-specific primers for HPV 16, 18 and 33 [20]. HPV DNA-negative cases were also tested with the type-specific primers for HPV 16 and 18. PCR of six cases was negative for all markers. Analyses were performed blindly. For further details see [15].

2.5. Confounders

Tobacco smoking is implicated as a risk factor for cervical cancer [21]. HSV-2 has not been found to have a role in the aetiology of cervical cancer in prospective studies [22], but HSV-2 was a correlate of HPV in a study by Smith and colleagues [23]. *C. trachomatis* and the analyzed HPV types were treated as possible confounders if they were not present in the joint effects.

2.6. Statistical analyses

2.6.1. Imputation

Seven values of smoking were missing (four of them cases). There are 128 different combinations that can impute (replace) the missing values of smoking by the value of smoker or non-smoker. One of the combinations must be correct. Odds ratios (OR) for the joint effects of interest were estimated using 128 differently imputed data sets and the non-imputed data set. The results among non-imputed data did not lie within the range of the results from the imputed data sets. Therefore it was necessary to impute the missing values of smoking. For each participant in whom information about smoking was lacking, a subset of more than 20 controls was formed, with age at and year of serum sampling centred on those of the particular participant. A random-number on interval [0,1] was generated. If the random number was smaller than the smoking prevalence in the control subset, the participant received smoker status. Otherwise, the participant received non-smoker status.

2.6.2. Regression

ORs were estimated by conditional logistic regression with *GLIM 4* software (Numerical Algorithms Group, Oxford, UK). 95% confidence intervals (CI) for the ORs are based on profile likelihood [24].

2.6.3. Expected joint effects

For Tables 2 and 4, in the calculus of the expected OR, OR_{m2}, for the multiplicative joint effect of two exposures, X and Y, ORs for the solitary effects, i.e. effects of alone-occurring exposures were used: $OR_{m2}(X,Y) = OR_{X-Y-Z}\ OR_{-XY-Z}$, in which ^{-}Y denotes those not exposed to Y. The joint effect of two exposures is called first-order interaction. The formula used to estimate the expected OR for the joint effect of three exposures, the second-order interaction, was $OR_{m3} = OR_{X-Y-Z}\ OR_{-XY-Z}\ OR_{-X-YZ}$. The expected OR for the additive joint effect was $OR_{a2}(X,Y) = OR_{X-Y-Z} + OR_{-XY-Z} - 1$ and similarly for three exposures, $OR_{a3} = OR_{X-Y-Z} + OR_{-XY-Z} + OR_{-X-YZ} - 2$. The formulae of OR_{m2} and OR_{a2} apply to Table 3 without Zs. The confidence limits for the expected multiplicative OR were approximated by the delta method on the logarithms of the OR estimates. The confidence limits for the expected additive OR were approximated by the delta method on the OR estimates.

Testing for multiplicative vs. non-multiplicative interaction of exposures was done with a likelihood ratio test to compare two nested models, one for the solitary effects only and the other for the solitary effects and the interaction/s, by considering the difference between the model-specific scaled deviances. The *P*-value for additive vs. non-additive interaction was obtained from the standardized normal distribution using $(OR_{XYZ}-OR_{a3})/se(OR_{XYZ}-OR_{a3})$, in which *se* is the standard error, as a test statistic for a two-sided test. The test statistic for Table 3 was $(OR_{XY}-OR_{a2})/se(OR_{XY}-OR_{a2})$.

2.6.4. Correction for misclassification

The sensitivity of HPV capsid serology has been found to be in the range of 50–75% and its specificity is likely to be at least 98% [25]. Effects of the misclassification of HPV serology were explored by assuming a 'gold standard' OR = 20 of SCC for HPV16. The 'gold standard' was based on comparable, system-

Table 2
Multivariate-adjusted[a] odds ratios for squamous cell carcinoma of the uterine cervix related to seropositivity for human papillomavirus (HPV) type 16 and/or 6/11, and/or *Chlamydia trachomatis (C. tr)*, in a cohort of 550 000 Nordic women

Seropositivity for			No. of		Observed 95% CI odds ratio	Expected interaction	
HPV16	HPV6/11	*C.tr.*	Cases	Controls		Multiplicative OR_m[b] 95% CI	Additive OR_a[c] 95% CI
No	No	No	62	306	1[d]		
Yes	No	No	19	12	8.1 (3.7, 19)		
No	Yes	No	17	37	2.3 (1.3, 4.1)		
No	No	Yes	29	38	3.1 (1.8, 5.3)		
1st order interactions							
Yes	Yes	No	3	8	2.3 (0.5, 8.2)	19 (5.7, 60)	9.4 (1.9, 17)
Yes	No	Yes	5	8	2.4 (0.7, 7.0)	25 (8.2, 76)	10.2 (2.7, 18)
No	Yes	Yes	8	7	3.7 (1.4, 10.3)	7.1 (2.8, 18)	4.4 (1.9, 6.9)
2nd order interaction							
Yes	Yes	Yes	1	4	1.0 (0.1, 6.3)	57 (14, 230)	11 (3.5, 19)

95% CI, 95% confidence interval; OR_a, expected additive odds ratio; OR_m, expected multiplicative odds ratio.
[a] Adjusted for herpes simplex virus type 2, HPV 18 and 33, and smoking.
[b] Product of the ORs for the solitary effects.
[c] Sum of the excess ORs for the solitary effects + 1.
[d] Reference category.

Table 3
Multivariate-adjusted[a] odds ratios for squamous cell carcinoma of the uterine cervix related to seropositivity for human papillomavirus (HPV) type 18/33 and/or 6/11, and/or *Chlamydia trachomatis* (*C. tr*), in a cohort of 550 000 Nordic women

Seropositivity for			No. of		Observed 95% CI odds ratio	Expected interaction	
HPV18/33	HPV6/11	*C.tr.*	Cases	Controls		Multiplicative OR_m[b] 95% CI	Additive OR_a[c] 95% CI
No	No	No	60	274	1[d]		
Yes	No	No	21	44	2.1 (1.2, 3.7)		
No	Yes	No	14	40	1.6 (0.8, 3.0)		
No	No	Yes	24	34	2.7 (1.5, 4.7)		
1st order interactions							
Yes	Yes	No	6	5	4.3 (1.3, 15)	3.4 (1.2, 9.2)	2.7 (0.9, 4.6)
Yes	No	Yes	10	12	3.1 (1.3, 7.3)	5.6 (2.2, 14)	3.8 (1.5, 6.0)
No	Yes	Yes	5	7	2.5 (0.7, 8.4)	4.3 (1.6, 12)	3.3 (1.2, 5.4)
2nd order interaction							
Yes	Yes	Yes	4	4	3.2 (0.8,13)	9.0 (2.5, 33)	4.4 (1.6, 7.1)

95% CI, 95% confidence interval; OR_a, expected additive odds ratio; OR_m, expected multiplicative odds ratio
[a] Adjusted for herpes simplex virus type 2, HPV 16, and smoking.
[b] Product of the ORs for the solitary effects.
[c] Sum of the excess ORs for the solitary effects + 1.
[d] Reference category.

atically reviewed follow-up studies with PCR diagnosis of HPV16 infection [26]. Also, other values, from 12 to 50, were applied for the 'gold standard' OR. Sensitivity analyses for non-differential misclassification and specific types of differential one were evaluated, as explained by Hakama and colleagues [7], in subgroups: HPV6/11 seronegatives, HPV6/11 seropositives, *C. trachomatis* seropositives, and *C. trachomatis* seronegatives.

3. Results

The OR of SCC for HPV16 seropositivity in the absence of HPV6/11 and *C. trachomatis* antibodies was 8.1 (95% CI 3.7–19), but HPV18/33 seropositivity was associated with a moderately increased risk only (Tables 2 and 3). HPV6/11 seropositivity and *C. trachomatis* seropositivity also showed moderately increased risks of SCC in the absence of the other antibodies.

In the above context the first-order interactions of HPV16 and HPV6/11, and HPV16 and *C. trachomatis*, were smaller than expected, i.e. antagonistic, assuming multiplicative and additive effects (Table 2). In contrast the first-order interactions of HPV18/33 and HPV6/11, and HPV18/33 and *C. trachomatis*, were close to that expected assuming multiplicative and additive effects (Table 3).

In general, there was almost no excess risk of SCC among women seropositive for HPV16 and HPV6/11, or for HPV16 and *C. trachomatis* (OR = 1.1, 95% CI 0.3–3.1, OR = 1.5, 95% CI 0.6–3.9; Table 4), although the expected multiplicative ORs were 8.7 and 15. The corresponding ORs of HPV16 DNA-positive SCC for the joint effects of HPV16 and HPV6/11, and HPV16 and *C. trachomatis* (OR, 1.9 and 1.7; Table 4), were also smaller than the expected multiplicative ORs, 22 and 32. No such antagonistic effects for SCC were observed for HPV18/33 and HPV6/11, and HPV18/33 and *C. trachomatis*. There were too few cases for analysis of HPV18/33 DNA-positive SCC.

The OR of SCC for the joint effect of HPV6/11 and *C. trachomatis* were close to expected ORs, and approximately additive in the absence of HPV16 antibodies as well as in the absence of HPV18/33 antibodies (Tables 2–4). The joint effect of HPV6/11 and *C. trachomatis* on the OR of HPV16 DNA-positive SCC was smaller than the expected multiplicative and additive effects, albeit not significantly (Table 4).

The second-order interaction of seropositivity for HPV16, HPV6/11 and *C. trachomatis* resulted in no

Table 4
Odds ratios (OR) with 95% confidence intervals (CI) for cervical squamous cell carcinoma (SCC) and human papillomavirus (HPV) type 16 DNA-positive cervical SCC related to seropositivity for HPV types 16, 6/11, and/or *Chlamydia trachomatis*, in a cohort of 550 000 Nordic women

Exposure		Interaction	SCC				HPV16 DNA-positive SCC			
			Cases	Controls	OR 95% CI	*P* value	Cases	Controls	OR 95% CI	*P* value
Adjusted for[a] and *C. trachomatis*										
HPV16	HPV6/11									
–	–		91	344	1[b]		37	163	1[b]	
–	+		25	44	2.0 (1.2, 3.4)		12	17	3.0 (1.3, 7.1)	
+	–		24	20	4.3 (2.2, 8.6)		16	12	7.2 (3.1, 18)	
+	+	Observed	4	12	1.1 (0.3, 3.1)		3	7	1.9 (0.4, 7.8)	
		Expected multiplicative			8.7 (3.2, 24)	0.0034			22 (4.7, 101)	0.0151
		Expected additive			5.3 (1.8, 8.8)	0.0173			9.2 (0.9, 18)	0.055
Adjusted for a and HPV6/11										
HPV16	*C. trachomatis*									
–	–		79	343	1[b]		31	159	1[b]	
–	+		37	45	3.0 (1.8, 4.8)		18	21	3.9 (1.9, 8.0)	
+	–		22	20	5.0 (2.6, 10.1)		16	13	8.3 (3.6, 21)	
+	+	Observed	6	12	1.5 (0.6, 3.9)		3	6	1.7 (0.4, 6.8)	
		Expected multiplicative			15 (5.6, 39)	0.0004			32 (7.5, 140)	0.0021
		Expected additive			6.9 (2.7, 11)	0.0064			11 (1.5, 21)	0.0398
Adjusted for[a] and HPV16										
HPV6/11	*C. trachomatis*									
–	–		81	318	1[b]		37	155	1[b]	
–	+		34	46	2.3 (1.4, 3.7)		16	20	3.2 (1.5, 6.8)	
+	–		20	45	1.7 (0.9, 2.9)		10	17	2.9 (1.2, 7.0)	
+	+	Observed	9	11	2.0 (0.8, 5.0)		5	7	1.8 (0.5, 5.9)	
		Expected multiplicative			3.7 (1.6, 8.9)	0.2965			9.3 (2.2, 39)	0.0513
		Expected additive			2.9 (1.2, 4.6)	0.1057			5.2 (0.8, 9.5)	0.0585

[a] Herpes simplex virus type 2, smoking, HPV 18 and 33.
[b] Reference category.

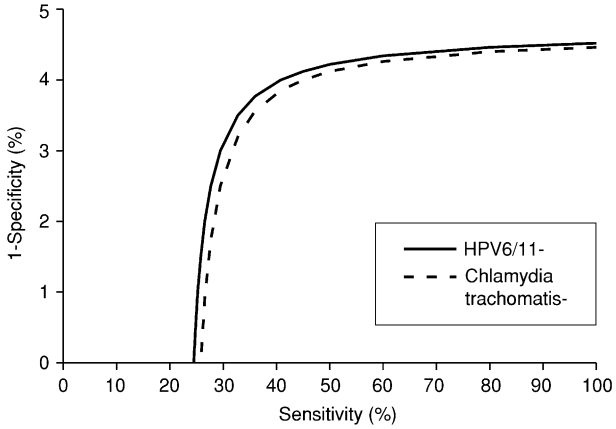

Fig. 1. Combinations of sensitivity and specificity consistent with the observed odds ratios (OR) of squamous cell carcinoma of the cervix uteri due to infection with human papillomavirus (HPV) type 16, 4.5 (among HPV6/11 negatives) or 4.8 (among *Chlamydia trachomatis* negatives), given the assumption that the true OR is 20.

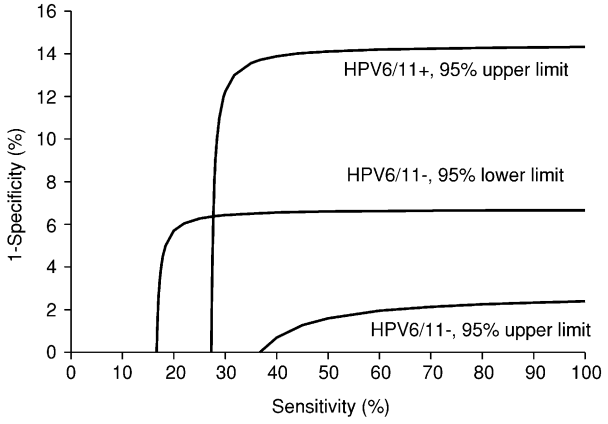

Fig. 2. Combinations of sensitivity and specificity consistent with the 95% confidence intervals of odds ratio for squamous cell carcinoma of the cervix uteri due to human papillomavirus (HPV) type 16, (2.4, 8.6) among HPV6/11 negatives and (0.2, 2.0) among HPV6/11 positives.

excess risk of SCC (OR = 1.0; Table 2). The observed OR was smaller than expected assuming either multiplicative (OR = 57, $P < 0.001$) or additive (OR = 11, $P = 0.011$) effects. In contrast, the OR for the joint effect of HPV18/33, HPV6/11 and *C. trachomatis*, 3.2, was close to the expected additive OR, 4.4 (Table 3).

In the sensitivity analyses the crude OR based on the frequencies in Table 4 of HPV16 in the subgroups defined by HPV6/11 and *C. trachomatis* were 0.6 (HPV6/11 seropositives, *C. trachomatis* seropositives), 4.5 (HPV6/11 seronegatives) and 4.8 (*C. trachomatis* seronegatives). No combinations of sensitivity and specificity in the HPV16 antibody assay would account for the difference between the observed 0.6 and the expected 'gold standard' OR, 20, among HPV6/11 seropositives and *C. trachomatis* seropositives (Fig. 1). For HPV6/11 seronegatives and *C. trachomatis* seronegatives, the HPV16-associated OR were not compatible with specificity values below 95% but were compatible with a wide range of sensitivities above 24% (Fig. 1). The 95% CI for the crude OR of HPV16 were 2.4–8.6 amongst HPV6/11 seronegatives, and 0.2–2.0 amongst HPV6/11 seropositives. The areas of sensitivity and specificity values consistent with the 95% CI among HPV6/11 seronegatives and seropositives overlapped only at very low sensitivities (Fig. 2). The corresponding figure for admissible areas among *C. trachomatis* seronegatives and seropositives was rather similar (not shown).

4. Discussion

An antagonistic joint effect of HPV16 and HPV6/11 on risk for cervical cancer has been independently estimated in two case-control studies [6,27]. An antagonistic interaction between HPV16 and *C. trachomatis* has also been found [7]. In the present study, an antagonistic second-order interaction was found between HPV16, HPV6/11 and *C. trachomatis* in the presence of an additive interaction between HPV6/11 and *C. trachomatis*. The second-order interaction was close to additive when HPV16 was replaced by HPV18/33.

Ours is, we believe, the first longitudinal study to elaborate the role of second-order interactions between three genital infections in cervical carcinogenesis. Due to histological reclassification [15], the study material differs from that of an earlier publication [6] by 12 cases for SCC. Confounding due to sexual risk-taking behaviour was now adjusted for by HSV-2 antibody positivity. Furthermore, we also stratified our analyses among HPV16 DNA-positive cases.

Reliable joint-effect estimates of second-order interactions require large materials. This is, we believe, the largest longitudinal study on cervical carcinogenesis with invasive cervical cancer as the endpoint, but it barely had the power to identify the observed antagonism. The antagonistic second-order interaction is based on a few observations: one case and four controls were seropositive for all the three agents: HPV16, HPV6/11 and *C. trachomatis*. However, the ratio of the triple seropositive cases to triple seropositive controls should increase 10-fold for the estimated interaction to be additive.

The method we used to correct for misclassification identifies the non-differential misclassifications in the HPV16 antibody assay. There were no plausible sensitivity and specificity combinations of that assay that could account for the difference between the observed OR for SCC associated with HPV16 antibodies and the corresponding 'gold standard' OR of between 12 to 50 among HPV6/11 seropositives and *C. trachomatis* seropositives. Thus true antagonism, differential misclassification or other sources of bias are plausible explanations for our observations.

The observed antagonistic interactions were specific for HPV16 but could still be pseudo-effects caused by differential misclassification. The method used to correct for misclassification does not rule out all types of differential misclassification bias [7]. Therefore, any biological conclusions are liable to error.

Depending on the chain (sequence) of events (infections), antagonistic responses might take place as follows: individuals who become first infected with HPV6/11, which alone has very low oncogenic potential (overruled by the host's normal immune response), may develop cross-protective cell-mediated immunity that is also protective against long-term sequelae of subsequent HPV16 infection, i.e. cervical cancer. Individuals who first become infected with HPV16, which alone has high oncogenic potential, are lacking such a cross-protective cell-mediated immunity. It has been suggested that *C. trachomatis* infection prolongs infection with HPV [28]. An additive interaction was observed between HPV6/11 and *C. trachomatis*. A prolonged infection would fit better with a theoretical multiplicative interaction, if HPV6/11 were carcinogenic. Provided that augmentation of the cross-reactive cell-mediated immune response to type-common early HPV antigens does take place during the prolonged HPV6/11 and *C. trachomatis* infection, and challenges the carcinogenic effect of a prolonged HPV16 infection, the observed antagonism between *C. trachomatis* and HPV16 could result. These mechanisms assume that HPV6/11 and *C. trachomatis* infections precede HPV16 infection.

Assuming that HPV6/11 infection preceding HPV16 protects absolutely from invasive cervical cancer and a random order of the two infections, the relative risk for the joint effect of HPV16 and HPV6/11 would be half of the relative risk for HPV16 in the absence of HPV6/11. The estimates of the relative risks for the joint effect were, however, less than half of those for the effect of HPV16 only. A possible explanation for this is that the women who had acquired persistent HPV16 infection before HPV6/11 infection may have received medical attention for genital warts caused by HPV6/11, and the persistent HPV16 infection (and associated precancerous lesions) was diagnosed at the same time and cured. This explanation is less plausible if HPV6/11 infection precedes HPV16 infection. The warts may have been cured before HPV16 was acquired or caused any lesions. Such a selective treatment results in selection in the material, as preinvasive lesions were not included in the present study.

It is possible that the increased relative risk among HPV6/11-seropositive HPV16 seronegatives is due to an unmeasured, oncogenic HPV infection. Thomas and colleagues [29] found that hazard ratios for acquiring oncogenic HPV45 after HPV6 as well as for acquiring HPV6 after HPV45 were increased. On the other hand, the identification of an increased relative risk for *C. trachomatis* in the absence of HPV16 is consistent with findings by Wallin and colleagues [9]. Prediagnostic smears of nine invasive cervical cancer cases were *C. trachomatis* DNA positive but HPV DNA-negative, and none of the matched controls was *C. trachomatis* DNA-positive. Hence, the estimate of relative risk of cervical cancer for *C. trachomatis* DNA was increased also among HPV DNA-negatives. How HPV16 could override the effects of HPV6/11 (or another oncogenic HPV type) or *C. trachomatis* remains open.

An exclusive and exhaustive explanation for the observed antagonistic interactions barely exists. We have discussed biological mechanisms for the observed antagonistic interaction between HPV16, HPV6/11 and *C. trachomatis*. Differential misclassification could cause antagonism, as could the selective (by infection) treatment of the women for premalignant and preinvasive lesions. We cannot say to what extent the observed antagonistic interactions are caused by immune reactions, differential misclassification of exposure, or selection by outcome.

Acknowledgements

The authors are indebted to Ms C. Eklund, Ms P. Kontiokari and Mr S. E. Sandlien for excellent technical assistance. Financial support was granted by the Academy of Finland, by the EU BIOMED-5 Concerted Action on Evaluation of the Role of Infections in Cancer using Biological Specimen Banks, by the King Gustav Vth Jubilee Fund, by the longitudinal epidemiology programme of the Nordic Council of Ministers, by the Nordic Cancer Union and by the Swedish Cancer Society. The Janus Serum bank owned by The Norwegian Cancer Society provided the Norwegian serum samples. This is publication no. 26 from the Nordic Biological Specimen Banks working group on Cancer Causes and Control.

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