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Original Contribution

Is Smoking an Independent Risk Factor for Invasive Cervical Cancer? A Nested Case-Control Study Within Nordic Biobanks

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The strong correlation between smoking and exposure to oncogenic human papillomaviruses (HPVs) has made it difficult to verify the independent role of smoking in cervical carcinogenesis. Thus, the authors evaluated this role. Five large Nordic serum banks containing samples from more than 1,000,000 subjects were linked with nationwide cancer registries (1973–2003). Serum samples were retrieved from 588 women who developed invasive cervical cancer and 2,861 matched controls. The samples were analyzed for cotinine (a biomarker of tobacco exposure) and antibodies to HPV types 16 and 18, herpes simplex virus type 2, and *Chlamydia trachomatis*. Smoking was associated with the risk of squamous cell carcinoma (SCC) among HPV16- and/or HPV18-seropositive heavy smokers (odds ratio = 2.7, 95% confidence interval: 1.7, 4.3). A similar risk of SCC (odds ratio = 3.2, 95% confidence interval: 2.6, 4.0) was found in heavy smokers after adjustment for HPV16/18 antibodies. The point estimates increased with increasing age at diagnosis and increasing cotinine level. This study confirms that smoking is an independent risk factor for cervical cancer/SCC in women infected with oncogenic HPVs. These findings emphasize the importance of cervical cancer prevention among women exposed to tobacco smoke.

carcinoma, squamous cell; risk factors; smoking; uterine cervical neoplasms

Abbreviations: CI, confidence interval; HPV, human papillomavirus; MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease; SCC, squamous cell carcinoma.

Invasive cervical cancer is among the leading causes of cancer mortality, predominantly in developing countries (1). Cervical infection with high-risk types of human papillomavirus (HPV) is the main cause of invasive cervical cancer. Of the numerous oncogenic HPV types, 70% of cervical cancer is attributed to HPV16 and HPV18 (2–4). However, infection with HPV16/18 cannot be a sufficient cause of cervical cancer (5) because of the high numbers of HPV16/18-infected women who do not develop cancer. Hence, the roles of other potential risk factors for cervical cancer need to be considered.

An increased risk of cervical cancer associated with tobacco smoking has been established on the basis of epidemiologic studies (6–8). Smoking is of interest as a cofactor in cervical carcinogenesis because of 1) the consistency and strength of the association of smoking with cervical intraepithelial neoplasia grade 3 and cervical cancer; 2) biologic plausibility, including the observation of nicotine-derived carcinogens in cervical mucus after smoking; and 3) the potential for intervention through antismoking campaigns.

A number of studies restricted to HPV DNA-positive women have shown an increased risk of cervical cancer in smokers compared with nonsmokers (9–11). However, the strong correlation between smoking and exposure to sexually transmitted diseases has made it difficult to verify the independent role of smoking in cervical carcinogenesis.

In previous studies, serum cotinine level was found to be a more precise measure of nicotine consumption than self-reported use of cigarettes (12, 13). Therefore, evaluation of the relation between serum cotinine level and cervical cancer risk might contribute to a better understanding of the quantitative aspects of tobacco-related cervical carcinogenesis.

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The construction of a joint Nordic cohort of more than 1,000,000 women offered us an opportunity to analyze the role of smoking, measured via serum cotinine level, in cervical carcinogenesis over time. Our specific aim was to distinguish whether the association between smoking and cervical cancer was due to an independent role of smoking in cervical cancer or due to residual confounding by oncogenic HPV types.

MATERIALS AND METHODS

Serum banks

Five population-based serum banks collaborated in this nested case-control study.

The Finnish Maternity Cohort was established in 1983. By 2006, it comprised approximately 1,300,000 serum samples collected from about 750,000 women—practically all pregnant women visiting maternity clinics during the first trimester of pregnancy. The samples are stored at −25°C at the National Public Health Institute in Oulu, Finland (14).

The Iceland Maternity Cohort, by 2003, contained approximately 96,000 serum samples collected from approximately 50,000 women at 12–14 weeks of pregnancy for rubella screening. The samples have been stored since 1980 in the Department of Medical Virology at Landspitali University Hospital, Reykjavik, Iceland (14).

The Northern Sweden Maternity Cohort consists of serum samples collected since 1975 from pregnant women screened for rubella immunity during week 14 of pregnancy in Västerbotten County and, especially in the 1980s, for some of the adjacent counties in northern Sweden. So far, almost 120,000 samples from approximately 86,000 women have been stored at the virus laboratory of Umeå University, Umeå, Sweden (14).

The Northern Sweden Health and Disease Study comprises the Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Project and the Västerbotten Intervention Program. The MONICA Project contains material from population-based screenings for cardiovascular disease that were carried out in 1986, 1990, 1994, 1999, and 2004. There were approximately 14,000 sampling occasions for approximately 9,000 persons, 50% of whom are included in the Västerbotten Intervention Program. Established in 1985, the Västerbotten Intervention Program is a long-term health promotion project that by 2004 included approximately 74,000 persons, of whom approximately 70,000 had donated blood. The samples are collected annually from all residents aged 40, 50, and 60 years in Västerbotten County and are stored as plasma at −80°C at the university hospital of the University of Umeå, Umeå, Sweden (14, 15).

The Janus Serum Bank was established in Oslo, Norway, in 1973 to identify early changes related to chronic disease development. In 2003, the Janus Serum Bank contained approximately 430,000 serum samples from 331,801 donors, 10% of them Red Cross donors. Approximately 145,000 women have been recruited during routine health examinations in subsequent phases. The samples were collected during 2 periods (1974–1978 and 1983–1991), with serum samples being stored at −25°C (14).

Population-based cancer registries

The cancer registries of Finland, Iceland, and Norway and the regional cancer registry in Umeå are all population-based and countrywide (4 northernmost Swedish counties for Umeå). Audits have shown that virtually all cases of cancer are being reported to the cancer registries by physicians and pathology/hematology laboratories, and 95% of them are histologically confirmed (16). Following approval by ethical committees, serum banks were linked to national cancer registries using the unique personal identification number assigned to all residents of Nordic countries.

Identification of cases and controls

We first performed a study (study 1) based on 171 identified cervical cancer cases matched to 496 unaffected controls. The objective was to elaborate the association between smoking and cervical cancer. We found an increased risk of cervical squamous cell carcinoma (SCC) among smokers, but because of its small sample size, study 1 had limited power to distinguish whether smoking was an independent cofactor in cervical carcinogenesis or its role was due to residual confounding by the oncogenic HPVs. In a second study (study 2), we assembled almost 4 times as much independent material, including 588 cervical cancer cases and 2,861 controls (Table 1).

Cases were women diagnosed with invasive cervical cancer following their enrollment in a cohort. Cases were identified by linking the serum bank data files with the national cancer registries. In study 1, 196 cases were identified. Four cases had too short a lag time from serum sampling to diagnosis. Ten cases were benign or lacked reported information on histologic type. Thereafter, the number of cervical cancer cases decreased from 182 to 178 because of histologic reclassification. Among those 178 cases, 7 did not have enough serum left for cotinine analysis after the other laboratory analyses had been conducted. In study 2, 653 cases were identified. The samples of 34 cases could not be located. The samples of 16 women were benign or without reported histologic type. Among the remaining 603 cervical cancer cases, 15 did not have enough serum left for all laboratory analyses.

Controls were selected from the same serum bank as cases (Table 1). For each case, 5 female controls who were alive and free of cancer at the time of the case's diagnosis were randomly selected and matched on age at serum sampling (±2 years), storage time (±2 months), and county in Norway. If 5 controls could not be found, the matching criteria of age at blood collection and length of frozen storage were widened.

The average time from the date of serum sampling to the date of diagnosis was 4.7 years in study 1 and 9.6 years in study 2. In study 1, mean ages at diagnosis were as follows: Finnish Maternity Cohort, 34 years; Janus health examinations, 47 years; Norway 1974–1978, 46 years; Norway 1981–1992, 47 years; and Northern Sweden Health and

Table 1. Characteristics of the Nordic Joint Cohort Created for a Nested Case-Control Study of Tobacco Smoke Exposure and Risk of Invasive Cervical Cancer, 1973–2003

Serum Bank	Geographic Location	Period of Serum Sampling	No. of Female Serum Sample Donors by End of 2003 (Rounded)	No. of Cases		No. of Controls		Age at Diagnosis, years	
				Study 1	Study 2	Study 1	Study 2	Study 1	Study 2
Janus Serum Bank	3 counties in Norway	1974–1978	29,000	79	36	237	178	26–63	32–70
	Several counties in Norway	1981–1992	115,000	47	129	141	639	35–63	29–67
	Red Cross blood donors in Oslo, Norway	1973–1991 and 1997–2000	14,000	—[a]	45	—	223	—	29–69
Northern Sweden Health and Disease Study (VIP and MONICA cohorts)	2 northernmost counties in Sweden	1985–present	38,000	4	—	12	—	39–60	—
Northern Sweden Maternity Cohort	4 northernmost counties in Sweden	1975–present	86,000	—	111	—	530	—	21–57
Finnish Maternity Cohort	Finland	1983–present	681,000	41	167	106	798	22–49	22–54
Icelandic Maternity Cohort	Iceland	1980–present	49,000	—	100	—	493	—	23–55
Total	Nordic countries	1973–present	1,012,000	171	588	496	2,861	22–63	21–70

Abbreviations: MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease; VIP, Västerbotten Intervention Program.
[a] Data not available.

Disease Study, 50 years. In study 2, the mean ages at diagnosis were: Finnish Maternity Cohort, 38 years; Janus health examinations, 49 years; Norway 1974–1978, 56 years; Norway 1981–1992, 47 years; Janus Red Cross blood donors, 44 years; Northern Sweden Maternity Cohort, 37 years; and Iceland Maternity Cohort, 34 years.

Laboratory methods

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

We measured cotinine using a qualitative immunoassay method (OraSure Technologies, Bethlehem, Pennsylvania) that is carried out as a quantitative assay and is based on the competition between free cotinine in the sample and cotinine bound to horseradish peroxidase-labeled cotinine. Cotinine concentration was quantified by measuring the light absorbance of wavelengths of 450 nm and 630 nm and by comparing the cotinine concentration of each sample with the standard curve. This assay has a sensitivity of 95%–97% and a specificity of 99%–100% (17–19). Careful testing of this method has revealed excellent correlations with established gas chromatography (20) and radioimmunoassay (21). Regression dilution bias was assessed by measuring paired samples repeatedly with the same batch from the assay kit (22).

The cotinine level that is accepted as defining an active smoker depends on the prevalence of smoking in the population (19, 23, 24). In our study, the measured cotinine levels were categorized into 3 groups: less than 20 ng/mL for nonsmokers or persons passively exposed to tobacco smoke and 2 other categories of 20–$\leq$100 ng/mL and $\geq$100 ng/mL, corresponding approximately to average levels found among light and heavy smokers.

The presence of immunoglobulin G antibodies specific for HPV types 16 and 18 was determined by means of a standard enzyme-linked immunosorbent assay. The assay employed baculovirus-expressed capsids comprising both the L1 and L2 proteins, with disrupted capsids of bovine papillomavirus used as the negative control. The specificity of HPV serology was found to be high, since no antibodies could be found in serum samples from virginal women and seropositivity had a linear relation to lifetime number of sexual partners (25). The sensitivity of HPV serology for detecting past HPV infection has been estimated to vary between 50% and 70% (26, 27).

The presence of immunoglobulin G antibodies specific for *Chlamydia trachomatis* was determined by the microimmunofluorescence method according to the manufacturer's instructions (28). Seropositivity to *C. trachomatis* shows a strong correlation with lifetime number of sexual partners (29).

The presence of immunoglobulin G antibodies to herpes simplex virus type 2 was also determined using a commercially available herpes simplex virus type 2 glycoprotein gG-2-based enzyme-linked immunosorbent assay (Biokit SA, Barcelona, Spain) according to the manufacturer's recommendation (30).

Table 2. Distribution of Cervical Cancer Cases and Controls According to Tobacco Smoke Exposure (Defined as Blood Cotinine Level) in a Nordic Joint Cohort of 1,012,000 Women, 1973–2003

Study and Cotinine Level, ng/mL	Invasive Cervical Cancer				Squamous Cell Carcinoma				Adenocarcinoma			
	Cases		Cases		Cases		Cases		Cases		Cases	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Study 1	171		496		141		412		23		64	
<20	84	49	294	59	63	45	234	57	16	70	46	72
20–<100	12	7	35	7	11	8	32	8	1	4	3	5
≥100	75	44	167	34	67	48	146	35	6	26	15	23
Study 2	588		2,861		445		2,167		115		557	
<20	306	52	1,937	68	210	47	1,468	68	80	70	386	69
20–<100	114	19	447	16	91	20	333	15	17	15	86	15
≥100	168	29	477	17	144	32	366	17	18	16	85	15

Statistical analyses

Relative risks, expressed as odds ratios, were estimated using conditional logistic regression for matched case-control sets of cervical cancer by means of GLIM4 (Numerical Algorithms Group, Oxford, United Kingdom) (31). The 95% confidence intervals for the odds ratios were based on profile likelihood (32). Heterogeneity in odds ratio estimates was assessed with a likelihood ratio test. The nested models compared included one for the overall effects of the cotinine groups (20–<100 ng/mL and ≥100 ng/mL) and another that also included serum bank-specific effects of the cotinine groups. Unconditional logistic regression was applied to HPV16/18-seropositive cases and controls, with the matching variables (serum bank, subcohort, storage time, and age at serum sampling) included in the model.

Increasing tobacco smoke exposure was defined according to blood cotinine levels. The cotinine levels used for evaluating the relation between cotinine dose and risk of cervical cancer in study 2 were quartiles among control women with cotinine levels greater than or equal to 20 ng/mL and the cutoff level for heavy smoking, which was close to the median, 102 ng/mL. In the dose-response analyses, the quartiles (cutpoints: 68 ng/mL, 100 ng/mL, and 140 ng/mL) of active smokers (≥20 ng/mL) were used. The dose-response relation was tested by likelihood ratio test and consisted of adding the numerical dose-response variable to the threshold model in which a variable indicated women whose cotinine level was at least 20 ng/mL. Likelihood ratio tests for linear trends in the logarithm of the odds ratio were performed for increasing age at diagnosis among women with cotinine levels of 20–<100 ng/mL and ≥100 ng/mL, with and without adjustment for HPV16/18, herpes simplex virus type 2, and *C. trachomatis*.

RESULTS

Distribution of cotinine levels in cases and controls

In study 1, 141 cases (82%) were SCC and 23 cases (13%) were adenocarcinoma. In study 2, 445 cases (76%) were SCC and 115 cases (20%) were adenocarcinoma (Table 2). Case women had elevated cotinine levels more often than did their matched controls. In study 1, 55% of SCC cases had a cotinine level of ≥20 ng/mL compared with 43% of the controls; and in study 2, 53% of the SCC cases had a cotinine level of ≥20 ng/mL compared with 32% of the controls. The proportion of women with a cotinine level greater than or equal to 20 ng/mL was higher among SCC cases than among adenocarcinoma cases. In study 2, the proportion of women with a cotinine level greater than or equal to 100 ng/mL was considerably higher than that of women with a cotinine level of 20–<100 ng/mL among the SCC cases, but not among the adenocarcinoma cases or controls (Table 2).

Odds ratios for cervical cancer by tobacco smoke exposure

Table 3 provides crude and adjusted smoking-associated odds ratios for cervical cancer, SCC, and adenocarcinoma in women with cotinine levels of 20–<100 ng/mL and ≥100 ng/mL. In both study 1 and study 2, a serum cotinine level greater than or equal to 100 ng/mL was associated with an increased risk of cervical cancer (odds ratios were 1.7 (95% confidence interval (CI): 1.2, 2.5) and 2.5 (95% CI: 2.0, 3.0), respectively). A cotinine level of ≥20 ng/mL was associated with an increased risk of SCC. In study 2, the HPV16/18-adjusted odds ratio for SCC was stronger in women with a cotinine level of ≥100 ng/mL (odds ratio = 3.2, 95% CI: 2.6, 4.0) than in women with a cotinine level of 20–<100 ng/mL (odds ratio = 2.2, 95% CI: 1.7, 2.8). Further adjustment for antibodies to HPV16/18, herpes simplex virus type 2, and *C. trachomatis* had no material effects on the point estimates (Table 3). Both studies also showed that serum cotinine level was not associated with an excess risk of cervical adenocarcinoma.

Odds ratios for cervical cancer by tobacco smoke exposure and HPV16/18 status

Because of an ample sample size, the analysis of study 2 was restricted to HPV16/18-seropositive and -seronegative cases and controls. Again the point estimates increased with increasing serum cotinine levels (Table 4). Among HPV 16/18-seropositive women, significantly increased odds

Table 3. Crude and Adjusted Odds Ratios for Cervical Cancer According to Tobacco Smoke Exposure (Defined as Blood Cotinine Level) in a Nordic Joint Cohort, 1973–2003

Study and Cotinine Level, ng/mL	Invasive Cervical Cancer					Squamous Cell Carcinoma					Adenocarcinoma				
	OR_1[a]	95% CI	OR_2[b]	95% CI	OR_3[c]	95% CI	OR_1[a]	95% CI	OR_2[b]	95% CI	OR_3[c]	95% CI	OR_1[a]	95% CI	OR_2[b]	95% CI	OR_3[c]	95% CI
Study 1	(171 cases)		(170 cases)		(170 cases)		(141 cases)		(140 cases)		(140 cases)		(23 cases)		(23 cases)		(23 cases)	
	(496 controls)		(490 controls)		(490 controls)		(412 controls)		(407 controls)		(407 controls)		(64 controls)		(64 controls)		(64 controls)	
<20	1		1		1		1		1		1		1		1		1	
20–<100	1.3	0.6, 2.5	1.1	0.5, 2.2	1.0	0.5, 2.1	1.3	0.6, 2.7	1.1	0.5, 2.2	1.0	0.5, 2.0	1.1	0.1, 13	1.2	0.1, 15	0.8[d]	0.0, 11
≥100	1.7	1.2, 2.5	1.6	1.1, 2.3	1.5	1.0, 2.1	1.8	1.2, 2.7	1.7	1.2, 2.6	1.6	1.1, 2.3	1.4	0.4, 4.5	1.1	0.3, 3.8	0.9[d]	0.2, 3.1
Study 2	(588 cases)		(588 cases)		(577 cases)		(445 cases)		(445 cases)		(437 cases)		(115 cases)		(115 cases)		(113 cases)	
	(2,861 controls)		(2,858 controls)		(2,776 controls)		(2,167 controls)		(2,166 controls)		(2,102 controls)		(557 controls)		(555 controls)		(542 controls)	
<20	1		1		1		1		1		1		1		1		1	
20–<100	1.7	1.4, 2.1	1.7	1.4, 2.1	1.5	1.2, 1.9	2.0	1.6, 2.5	2.2	1.7, 2.8	1.9	1.5, 2.4	1.0	0.6, 1.6	0.9	0.6, 1.5	0.9	0.5, 1.5
≥100	2.5	2.0, 3.0	2.5	2.0, 3.0	2.2	1.8, 2.7	3.2	2.6, 4.0	3.2	2.6, 4.0	2.9	2.3, 3.6	1.0	0.6, 1.7	1.0	0.6, 1.6	1.0	0.6, 1.6
P value[e]	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		0.61		0.56		0.55	

Abbreviations: CI, confidence interval; HPV, human papillomavirus; OR, odds ratio.

[a] Crude OR.

[b] Adjusted for antibodies to HPV types 16 and 18.

[c] Adjusted for antibodies to HPV16 and HPV18, herpes simplex virus type 2, and *Chlamydia trachomatis*.

[d] One variable indicating seropositivity for HPV type 16 and/or 18.

[e] Test for dose-response relation (see "Statistical analyses").

ratios for cervical cancer and SCC were noted in those with a cotinine level greater than or equal to 100 ng/mL (odds ratios were 2.1 (95% CI: 1.4, 3.2) and 2.7 (95% CI: 1.7, 4.3), respectively). Adjustment for antibodies to herpes simplex virus type 2 and *C. trachomatis* had no effect on the point estimates. Among women with a cotinine level of 20–<100 ng/mL, the point estimates lost statistical significance. In HPV16/18-seronegative women, increased risks of cervical cancer and SCC were observed in both women with cotinine levels of 20–<100 ng/mL and women with cotinine levels of ≥100 ng/mL. We found a dose-response relation between cotinine level and the risk of cervical cancer in both HPV16/18-seropositive women and HPV16/18-seronegative women (Table 4).

Odds ratios for SCC by tobacco smoke exposure and age at diagnosis

Finally, the smoking-associated crude and adjusted odds ratios for SCC were estimated by stratifying for cases' age at diagnosis. In study 2, a dose-response effect was observed: The risk of SCC in women with cotinine levels of 20–<100 ng/mL increased with age after age 30 years, and the point estimates in women aged 30–<40, 40–<50, and ≥50 years with cotinine levels greater than or equal to 100 ng/mL increased with age at diagnosis in both studies (Table 5). The tests for linear trend were significant both among women with cotinine levels of 20–<100 ng/mL and among women with cotinine levels of ≥100 ng/mL, with and without adjustment for antibodies to HPV16/18, herpes simplex virus type 2, and *C. trachomatis* (all P's < 0.001).

DISCUSSION

We identified smoking as an independent risk factor for invasive cervical cancer. Not only was smoking associated with an increased risk of cervical cancer/SCC after adjustment for antibodies to oncogenic HPVs, but notably a significantly increased 2-fold excess risk of SCC, free of residual confounding bias, was found among HPV16/18-seropositive women. We also noted that the risk of SCC increased with increasing cotinine dose and age at diagnosis.

The Nordic joint study (14) is by far the largest nested case-control study on risk factors for cervical cancer to have been conducted to date. Because of its sample size and the longitudinal study design, it has verified or falsified a number of associations between the disease and its risk factors: oncogenic HPVs, *C. trachomatis*, retinoids (25, 29, 33), and herpes simplex virus type 2 (30). In the present study, our aim was to assess whether the association between smoking and cervical cancer was due to an independent role of smoking in cervical carcinogenesis or due to residual confounding by the oncogenic HPV types.

Questionnaire data on smoking history would have permitted us to assess lifetime exposure to tobacco smoke and to study the effects of smoking in relation to age at starting and stopping smoking. However, this method underestimates true prevalence (19) and is inaccurate with regard to

Table 4. Crude and Adjusted Odds Ratios for Cervical Cancer According to Human Papillomavirus Type 16 and/or Type 18 Status and Tobacco Smoke Exposure (Defined as Blood Cotinine Level) in a Nordic Joint Cohort, 1973–2003

HPV16/18 Status and Cotinine Level, ng/mL	Invasive Cervical Cancer				Squamous Cell Carcinoma			
	Crude OR	95% CI	Adjusted OR[a]	95% CI	Crude OR	95% CI	Adjusted OR[a]	95% CI
HPV16/18-seropositive women	(253 cases) (597 controls)		(247 cases) (589 controls)		(202 cases) (460 controls)		(197 cases) (452 controls)	
<20	1		1		1		1	
20–<100	1.1	0.7, 1.8	1.1	0.7, 1.8	1.2	0.7, 2.0	1.1	0.6, 2.0
≥100	2.1	1.4, 3.2	2.1	1.4, 3.2	2.7	1.7, 4.3	2.7	1.7, 4.4
P value[b]	<0.001		<0.001		<0.001		<0.001	
HPV16/18-seronegative women	(335 cases) (2,261 controls)		(330 cases) (2,234 controls)		(243 cases) (1,706 controls)		(240 cases) (1,686 controls)	
<20	1		1		1		1	
20–<100	2.0	1.6, 2.6	1.8	1.4, 2.3	2.7	2.0, 3.6	2.2	1.7, 3.1
≥100	2.6	2.0, 3.4	2.3	1.8, 3.0	3.4	2.5, 4.5	2.9	2.2, 4.0
P value[b]	0.04		0.05		0.06		0.06	

Abbreviations: CI, confidence interval; HPV, human papillomavirus; OR, odds ratio.
[a] Adjusted for herpes simplex virus type 2 and *Chlamydia trachomatis*.
[b] Test for dose-response relation (see "Statistical analyses").

smoking exposure. On the other hand, it is the inhaled dose of tobacco smoke that is directly related to the development of tobacco-related diseases (18). Biochemical assessment integrates different aspects of true exposure, including tobacco composition, uptake, and distribution and individual differences in metabolism. Cotinine is likely to be a good marker of intake of nicotine, which is the important carcinogen in tobacco smoke. Its serum level has a high sensitivity and specificity for tobacco exposure, and the measurement error is negligible in comparison with questionnaires (13, 19). In addition, although serum cotinine measures recent exposure to tobacco smoke, near the time of specimen collection, cotinine levels remain stable in frozen samples (13), permitting identification of the dose-response effect.

The hypothesis that smoking is a risk factor for cervical cancer was first put forward in 1977 by Winkelstein (34). Subsequent epidemiologic studies confirmed the hypothesis and reported strong evidence for an association of smoking with the risk of cervical SCC but not cervical adenocarcinoma. An increased risk was especially associated with a decreased age at starting smoking (7, 35).

HPV infection is such a powerful risk factor for cervical cancer that control of its effects is critical in the epidemiologic assessment of etiologic cofactors. In our longitudinal study, significantly increased risk estimates were found in heavy smokers after adjusting for antibodies to oncogenic HPV types. Our results are in line with previous cross-sectional studies (36–38) in which, after adjustment for HPV DNA, heavy or current smoking was associated with an increased risk of SCC or cervical cancer.

The observed effect of smoking could, however, be confounded by misclassification bias in the serologic diagnosis of high-risk HPV infection. Therefore, we carried out stratified analyses to confirm the independent role of smoking among HPV16/18-infected women. HPV virus-like particle serology has been proven to be a specific albeit nonsensitive marker of current and past exposure to HPV (39). It permits the delineation of HPV16/18-infected (seropositive) controls who could be compared with HPV16/18-infected (seropositive) cases. In addition, the assay was reproducible in the 2 studies (studies 1 and 2). In several recent studies (7), the analyses were restricted to HPV DNA-positive women. Most of these studies, however, test for HPV DNA at only 1 time point, which favors the detection of amplified HPV DNA in the cases. Furthermore, controls testing HPV-positive just once most likely have a transient infection. This could bias the measure for lifetime exposure and the impact of HPV infection. Such a problem does not exist for HPV-seropositive women, since the possibility that HPV antibodies wane over time is highly unlikely (40).

Our large longitudinal study enabled us to compare stratified and adjusted analyses. We observed highly significantly increased risks of cervical SCC for heavy smokers among both HPV16/18-seropositive women and HPV16/18-seronegative women (odds ratios were 2.7 and 2.9, respectively) and overall following adjustment for HPV16 and HPV18 antibodies (odds ratio = 3.2). Smoking was not associated with HPV antibody prevalence among the controls. Our results are consistent with the few prospective studies that have controlled for HPV infection using high-quality HPV assays. Deacon et al. (41) analyzed risk factors for progression to cervical intraepithelial neoplasia grade 3 among HPV-positive women in a nested case-control study and reported a high risk of 2.2 (95% CI: 1.4, 3.4) for ever smoking, with strong evidence of a dose-response effect.

We also found proportional increases in the risk estimates with increasing age at diagnosis of SCC and with increasing cotinine level. The effects of smoking, assessed by cotinine

Table 5. Crude and Adjusted Odds Ratios for Squamous Cell Carcinoma According to Cases's Age at Diagnosis and Tobacco Smoke Exposure (Defined as Blood Cotinine Level) in a Nordic Joint Cohort, 1973–2003

Study and Cotinine Level, ng/mL	Case's Age at Diagnosis, years															
	<30				30–<40				40–<50				≥50			
	OR_1[a]	95% CI	OR_2[b]	95% CI	OR_1[a]	95% CI	OR_2[b]	95% CI	OR_1[a]	95% CI	OR_2[b]	95% CI	OR_1[a]	95% CI	OR_2[b]	95% CI
Study 1	(10 cases)		(10 cases)		(31 cases)		(30 cases)		(63 cases)		(63 cases)		(37 cases)		(37 cases)	
	(26 controls)		(26 controls)		(89 controls)		(86 controls)		(186 controls)		(184 controls)		(111 controls)		(111 controls)	
<20	1		1		1		1		1		1		1		1	
20–<100	1.9	0.2, 13	0.7[c]	0.1, 5.4	0.7	0.1, 2.6	0.4	0.1, 1.9	1.9	0.5, 6.4	1.8	0.5, 6.2	1.5	0.3, 5.9	1.0	0.2, 4.3
≥100	1.3	0.2, 7.8	1.3[c]	0.2, 8.2	1.1	0.4, 2.8	1.1	0.4, 3.2	2.0	1.1, 3.5	1.8	1.0, 3.3	2.4	1.1, 5.4	2.2	1.0, 5.1
Study 2	(49 cases)		(49 cases)		(169 cases)		(165 cases)		(156 cases)		(153 cases)		(71 cases)		(70 cases)	
	(237 controls)		(235 controls)		(816 controls)		(785 controls)		(762 controls)		(734 controls)		(352 controls)		(346 controls)	
<20	1		1		1		1		1		1		1		1	
20–<100	1.0	0.5, 1.9	0.9	0.5, 1.7	1.9	1.3, 2.7	1.8	1.2, 2.6	2.7	1.7, 4.1	2.5	1.6, 3.9	2.7	1.3, 5.4	2.8	1.4, 5.7
≥100	1.4	0.6, 3.0	0.9	0.4, 2.0	2.9	1.9, 4.4	2.6	1.6, 4.0	3.4	2.4, 4.8	3.1	2.2, 4.4	5.0	3.1, 8.2	4.6	2.8, 7.6
P value[d]	0.93		0.47		0.02		0.06		0.08		0.12		0.03		0.03	

Abbreviations: CI, confidence interval; HPV, human papillomavirus; OR, odds ratio.

[a] Crude OR.

[b] Adjusted for antibodies to HPV16 and HPV18, herpes simplex virus type 2, and *Chlamydia trachomatis*.

[c] One variable indicating seropositivity for HPV types 16 and/or 18 and/or herpes simplex virus type 2 and/or *C. trachomatis*.

[d] Test for a dose-response relation (see "Statistical analyses").

level, did not depend on time from serum measurement to diagnosis. There is a strong dose-response effect between serum cotinine level and the risk of developing lung cancer (13), but this has not previously been described for cervical cancer. Recently, a pooled analysis of International Agency for Research on Cancer case-control studies (7) showed a significantly increased risk of cervical cancer for ever smokers over age 45 years, but the point estimates lost statistical significance in women under age 35 years. In a United Kingdom case-control study, the risk of SCC was significantly increased in long-term (≥20 years) smokers (35). Although young women appear to be heavy smokers, the carcinogenic effect of smoking is usually observed after a long period of exposure to tobacco smoke (42).

The presence of tobacco carcinogens in cervical mucus has been described as a possible biologic explanation for the epidemiologic association (43). The mechanism could involve soluble carcinogens that may have a direct transforming effect on squamous cervical epithelium. Some other epithelial cancers—those of the nasal cavity and esophagus—show similar differences between SCC and adenocarcinoma with regard to smoking and HPV (44–46). Cigarette smoking may also exacerbate the carcinogenic potential of HPV, specifically via inhibition of interferon-γ and/or tumor necrosis factor-α, leading to a significant inhibition of apoptosis, which may promote tumor growth (47). The fact that some cigarette constituents have the ability to manipulate cytokine expression in a manner similar to that of HPV suggests that smoking may enhance the ability of HPV to evade the immune system (48).

The population representativeness of the serum bank cohorts was recently assessed by Pukkala et al. (14), who found no significant difference in cancer incidence in the biobank cohorts as compared with respective national rates. These results could possibly be generalized to all Nordic women, since there was no evidence that the odds ratios among serum banks were heterogeneous.

In conclusion, smoking is an independent risk factor for cervical cancer/SCC. Seropositive women with antibodies to oncogenic HPVs who smoke are at significantly increased risk of developing cervical SCC. The rapid increase in smoking among young women may have a profound impact on future incidence of cervical cancer, emphasizing the need for joint preventive efforts.

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