

Sexual behaviour and papillomavirus exposure in cervical intraepithelial neoplasia: a population-based case-control study

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Sexual history is an established risk determinant for cervical neoplasia. It is not clear if human papillomavirus (HPV) exposure entirely explains the sexual behaviour-related risk or if other sexually transmitted agents may act as cofactors for HPV in carcinogenesis. The aim of this study was to elucidate whether HPV exposure or HPV persistence explains the sexual history-related risk of high-grade cervical intraepithelial neoplasia (CIN) using a population-based case-control study of most of the 254 women referred to colposcopy in the Västerbotten county in Sweden because of an abnormal cervical smear during October 1993 to December 1995 and 320 age-matched women from the general population. The women were interviewed for sexual history and tested for presence of serum antibodies to HPV-16, -18 and -33 as well as for presence of HPV DNA in cervical brush samples. HPV-16, -18 and -33 seropositivity was specific for the corresponding type of HPV DNA, dependent on the lifetime sexual history and associated with a two- to threefold increased risk of CIN 3. There was no sexual history-related risk of CIN among HPV-seropositive women and adjustment for HPV DNA presence explained the sexual history-related risk of CIN. In conclusion, HPV exposure appeared to explain the sexual history-related risk of high-grade CIN.

Introduction

Cervical cancer is one of the most common forms of cancer of women in the world, with an estimated 465 000 new cases and 200 000 deaths every year (Parkin *et al.*, 1988). Sexual behaviour is an established risk factor for cervical cancer and its precursor, cervical intraepithelial neoplasia (CIN) (Brinton *et al.*, 1987).

Sexually transmitted human papillomaviruses (HPV), especially types 16 and 18, are established as a major cause of

cervical cancer and CIN (Schiffman *et al.*, 1993). In some studies, presence of cervical HPV DNA has entirely explained the sexual behaviour-related risk of CIN (Schiffman *et al.*, 1993). However, a large body of literature has implicated other sexually transmitted agents as risk factors for cervical cancer, notably herpes simplex virus (Hildesheim *et al.*, 1993; de Sanjosé *et al.*, 1994; Becker *et al.*, 1994; Stone *et al.*, 1995), *Chlamydia trachomatis* (Dillner *et al.*, 1994; Munoz *et al.*, 1993; Hakama *et al.*, 1993) and even *Trichomonas vaginalis* (Slattery *et al.*, 1989; Yap *et al.*, 1995). In several of these studies, it has not been possible to explain the effect of these STDs because of confounding of HPV status, suggesting that some of these STDs may act as co-factors of HPV exposure in carcinogenesis.

The vast majority of HPV infections are transient, with

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about 70% of infections being cleared within 1 year (Hildesheim *et al.*, 1994; Evander *et al.*, 1995; Schiffman, 1995; Ho *et al.*, 1998). In the minority of subjects where the HPV infection persists, high-grade CIN and cervical cancer may develop. The factors that determine which of the HPV-exposed women go on to develop cancer is the major epidemiological enigma regarding cervical cancer aetiology today. One possible mode of action whereby other STDs could act as cofactors is by impairing the ability to clear HPV infection, perhaps by causing alterations in local immunity and thus increasing the probability of HPV infections becoming persistent. A problem with the studies that have found no residual sexual behaviour-associated CIN risk among HPV DNA-positive women is that women with current infection (recent exposures, plus a subset of old exposures that have become persistent) are studied, thus leaving the possibility open that other STDs may act as cofactors before HPV infection becomes persistent.

To elucidate this possibility, it would be necessary to analyse also the sexual behaviour-associated risk of CIN among women who have been exposed to HPV, but where the infection might have been cleared.

The standard HPV serological assay, based on HPV capsids, has been extensively validated as a marker of cumulative HPV exposure (past or present exposures). The sensitivity for HPV infection, using detection of viral DNA as reference, has been found to be about 50% (Chua *et al.*, 1996; de Gruijl *et al.*, 1997; Dillner *et al.*, 1996; Kirnbauer *et al.*, 1994; Wideroff *et al.*, 1995). The specificity for the sexually transmitted HPV types seems to be high, since no virginal and few monogamous women are seropositive (Andersson-Ellström *et al.*, 1996; Dillner *et al.*, 1996; Wideroff *et al.*, 1996). Presence of cervical HPV DNA is usually transient (Schiffman *et al.*, 1995; Evander *et al.*, 1995; Hildesheim *et al.*, 1994), is correlated with recent partner change (Hildesheim *et al.*, 1993) and shows declining prevalence with age (Melkert *et al.*, 1993). In contrast, presence of HPV antibodies in serum is also found after clearance of viral DNA (Wikström *et al.*, 1995; Carter *et al.*, 1996), the antibody levels persist on prolonged follow-up (af Geijersstam *et al.*, 1998) and there is no consistent association of HPV seropositivity with age (Chua *et al.*, 1996; Dillner *et al.*, 1996; Wikström *et al.*, 1995; Dillner *et al.*, 1997). Also, HPV seropositivity is not related to recent partner change, but strongly dependent on lifetime number of sexual partners (linear increase of seroprevalences with about 4% per partner) (Dillner *et al.*, 1996; Wideroff *et al.*, 1996), arguing that seroprevalences are preferable as markers of lifetime cumulative HPV exposure (Olsen *et al.*, 1997).

Therefore, studies on whether the sexual behaviour-related risk of CIN is explained by HPV exposure and/or by persistent HPV infection could help elucidate the aetiology of cervical cancer. We performed a population-based case-control study of high-grade CIN and sexual history, taking both HPV seropositivity and cervical HPV DNA positivity into account.

Methods

■ **Study population.** The Västerbotten county in Northern Sweden has 257 079 inhabitants (1993), of whom 128 834 are women. The population-based cervical screening programme started in 1969 and all women resident in the county aged 25–59 years are invited by letter for screening every 3 years. The participation rate is about 80%. The mean number of women living in this area and aged between 25 and 59 years of age was about 57 000. This study took place between October 1993 and March 1996. During this time, 87 892 cervical smears were taken, 33 072 (37.6%) in the organized screening programme and 54 820 (62.4%) outside the programme.

■ **Screening procedure.** The women are invited to their regional health care centre triannually. All cytological samples are taken by midwives, who prepare the slides for the standard Papanicolaou (Pap) staining procedure. The samples are examined by a cytology assistant in a single laboratory (the laboratory of Clinical Cytology, Umeå University Hospital) and if any pathological cells are found, the slide will be reviewed and classified by a senior cytologist. The cytological classification adheres to the classification criteria formulated by Koss (1979).

■ Study groups

Referral group. Between October 1993 and December 1995, 254 women with a pathological cervical smear were referred for colposcopy in Västerbotten county. One hundred and seventy-four women were referred from the population-based cervical screening programme and 76 because of smears taken outside the programme. They were referred to any of the three hospitals in Västerbotten county: Umeå University Hospital, Skellefteå Community Hospital or Lycksele Community Hospital. Four of the referred women refused to participate in the study, and the remaining 250 were included in the study.

Screening group. Women who were invited by letter for screening in the organized programme were asked via an additional enclosed letter to attend the study. These women were selected randomly from the population registry and matched to the referrals 1:1 for age \pm 3 years and area of residence. From October 1995 until March 1996, out of 2191 invited women, 871 were asked to participate and 320 accepted. In order to find out the reason why 63% did not accept the invitation, a randomly selected subgroup of 50 of these women was interviewed by telephone by the same investigator. Two were not interested in participating in the screening programme at all. The other 48 women had recently seen or intended to see a doctor or midwife for screening and did not want to participate because of inconvenience.

■ **Sampling procedures.** In the referral group, all women had been recruited either from the population-based invitational cervical screening programme or because of opportunistic screening. In January 1995, the organized screening programme switched the sampling instrument to a Cervex brush (Cortec Medical) that permits collecting cell samples from both ecto- and endocervix. The 114 samples that were taken before that time were taken with a cotton-tipped pin for the endocervix sample and a wooden spatula for the ectocervix and posterior fornix. The sample was prepared for standard Pap procedure and stored in 95% alcohol solution for transportation to the cytology laboratory. Before colposcopic examination, a sample was taken from the endocervix using a rotary motion with a Cytobrush (Medscand) for detection of cervical HPV DNA by nested PCR with consensus primers and type-specific primers for HPV-11, -16, -18 and -33. The sample for HPV assay was stored in physiological saline containing 10 mM Tris-HCl buffer, pH 7.8 and kept at -70°C until analysis. If colposcopy indicated a pathological finding, a punch biopsy was taken for histopathological diagnosis and if examination was normal an annual follow-up was arranged. If it was

Table 1. Presence of HPV antibodies in relation to cervical HPV DNA

Antibodies against	HPV DNA type	No. of women	Positive women (%)	OR	CI (95%)
HPV-16	Negative	376	26	1.0	—
	Other type	112	30	1.2	0.8–2.0
	HPV-16	52	65	5.3	2.8–10.5
HPV-18	Negative	376	16	1.0	—
	Other type	151	23	1.5	0.9–2.5
	HPV-18	13	69	11.5	3.1–52.8
HPV-33	Negative	376	16	1.0	—
	Other type	160	21	1.3	0.8–2.2
	HPV-33	4	75	15.3	1.2–81.6

obvious dysplasia, conization by a carbon dioxide laser was usually done immediately, otherwise after histopathological verification of dysplasia.

In the screening population, all women were examined by the same physician. Samples for cytomorphology and HPV DNA detection were taken and stored in the same manner as for referrals after January 1995. Although the sample for HPV DNA detection was taken identically for all cases and controls, the fact that the 114 cases sampled 1993–1994 had had their previous Pap smear taken with a different utensil might have introduced differential misclassification bias. This was investigated by comparing the HPV results for cases sampled before and after January 1995. No differences were found (Kjellberg *et al.*, 1998).

■ PCR analysis. All laboratory analyses were conducted with the analysing laboratory blind to the identity of samples. DNA from cells was prepared as previously described (Evander & Wadell, 1991). HPV DNA was amplified by two general primer pairs within the L1 open reading frame. The MY 11/09 consensus primer pair was used in combination with the GP 5/6 general primer pair in a nested, general primer, two-step amplification (Evander *et al.*, 1992; Manos *et al.*, 1989; Snijders *et al.*, 1990). First, 0.2 pmol MY 11/09 primer pair was mixed with PCR buffer (SDS/Promega), 3 mM MgCl₂, the four deoxy-nucleotides (100 mM of each), 1 U *Taq* polymerase (SDS/Promega) and 5 µl DNA prepared from cell samples. In total, 20 cycles were performed. The GP 5/6 primer pair (20 pmol) was then added to the reaction mixture together with 1.5 µl *Taq* polymerase, and 30 additional cycles were performed.

All specimens were also amplified with the β -globin primers GH 20 and PCO4 (Bauer *et al.*, 1991). Samples that were negative in the β -globin amplification were extracted with phenol, precipitated with ethanol, and repeat β -globin and HPV PCRs were performed. In each PCR reaction, every fifth sample contained water as a negative control, and all such controls were negative. DNA from the HPV-negative A549 cell line was also amplified with every batch of DNA from cervical cells and was always negative for HPV DNA and positive for β -globin DNA.

■ Detection and HPV-typing. Forty percent of the amplified DNA (20 µl) was separated on an agarose gel (2% NuSieve GTG plus 1.0% ScaKem ME; FMC Bioproducts) by electrophoresis and stained with ethidium bromide. The HPV-typing was performed by PCR using type-specific primers for HPV-11, -16, -18 and -33 (Evander & Wadell, 1991). In the event of a positive test for a type-specific HPV DNA, no further

testing was performed. For 91/168 HPV DNA-positive samples, the type was not one of the four types tested for (Kjellberg *et al.*, 1998).

■ Antibody analysis. The standard direct ELISA method (Kirnbauer *et al.*, 1994) for detecting IgG antibodies specific for HPV-16 using baculovirus-expressed capsids comprising both the L1 and L2 proteins was used, with disrupted capsids of bovine papillomavirus as negative control, as described in detail elsewhere (Heino *et al.*, 1995). The assays included several internal standard samples on each ELISA plate, both positive controls (pools of serum from patients with cervical dysplasia or cancer and from healthy women with cervical HPV-16 DNA) and negative controls (sera from sexually inexperienced women). The absorbance values from each plate were normalized relative to the results of the internal standard sera. The cut-off levels used were preassigned and, relative to internal standards, the same as used in previous studies (Andersson-Ellström *et al.*, 1996).

■ Statistical analysis. Conditional logistic regression analysis was used to calculate the odds ratio (OR). Exact confidence intervals for the OR were calculated using the permutational distribution of the sufficient statistics.

■ Ethical considerations. The study was approved by the institutional review board of Umeå University, decision number 93-197.

Results

Within-study validation of the serological assay regarding sensitivity, specificity and cross-reactivity between HPV types

In women testing positive for HPV-16, -18 or -33 DNA in cervical samples, a serum antibody response to HPV capsids of the corresponding HPV type was found among 65%, 69% and 75%, respectively (Table 1). The proportion of seropositive women among the women positive for cervical HPV DNA of another HPV type was significantly less and about the same as the proportion of seropositive women among women testing negative for cervical HPV DNA (Table 1). To evaluate whether the presence of serum HPV antibodies among HPV DNA-negative women was non-specific or due to previous type-specific HPV infections, the HPV seroprevalences were

Table 2. Presence of HPV antibodies among HPV DNA-negative women in relation to sexual history

No. of lifetime partners	No. of women	HPV-16			HPV-18			HPV-33		
		Positive women (%)	OR	CI (95%)	Positive women (%)	OR	CI (95%)	Positive women (%)	OR	CI (95%)
0-1	70	7	1.0	—	1	1.0	—	1	1.0	—
2-5	172	31	5.9	2.2-19.9	15	11.7	1.8-488	13	10.6	1.7-446
5+	122	31	5.8	2.1-20.1	28	26.4	4.2-1098	20	28.6	4.6-1190

Table 3. Presence of HPV antibodies in relation to presence of cervical lesions

(a) Presence of HPV antibodies in relation to cytological diagnoses

Cytological diagnoses	No. of women	HPV-16			HPV-18			HPV-33			HPV-16, -18, -33		
		Positive women (%)	OR	CI (95%)	Positive women (%)	OR	CI (95%)	Positive women (%)	OR	CI (95%)	Positive women (%)	OR	CI (95%)
Normal	302	26	1.0	—	16	1.0	—	14	1.0	—	38	1.0	—
ASCUS	71	35	1.6	0.9-2.8	14	0.8	0.4-1.8	17	1.3	0.6-2.7	46	1.4	0.8-2.4
HPV only	31	35	1.6	0.7-3.6	26	1.8	0.7-4.5	23	1.9	0.6-4.8	52	1.7	0.8-3.9
CIN suspect	75	27	1.0	0.6-1.9	17	1.1	0.5-2.2	24	2.0	1.0-3.9	41	1.1	0.7-1.9
CIN NOS	12	58	4.0	1.1-16.5	25	1.7	0.3-7.2	42	4.5	1.1-17.4	75	4.8	1.2-28.0
CIN 2-3	51	47	2.5	1.3-4.9	39	3.3	1.7-6.6	25	2.2	1.0-4.6	71	3.8	1.9-7.9

(b) Presence of HPV antibodies in relation to histological/cytological diagnoses

Histological/cytological* diagnoses	No. of women	HPV-16			HPV-18			HPV-33			HPV-16, -18, -33		
		Positive women (%)	OR	CI (95%)	Positive women (%)	OR	CI (95%)	Positive women (%)	OR	CI (95%)	Positive women (%)	OR	CI (95%)
Normal	348	26	1.0	—	17	1.0	—	14	1.0	—	39	1.0	—
HPV only	29	21	0.7	0.2-1.9	24	1.6	0.5-4.0	21	1.6	0.5-4.4	41	1.1	0.5-2.6
CIN 1	27	26	1.0	0.3-2.5	15	0.9	0.2-2.6	22	1.8	0.6-4.9	44	1.3	0.5-3.0
CIN 2	38	32	1.3	0.6-2.8	11	0.6	0.1-1.7	24	1.9	0.8-4.5	53	1.8	0.9-3.7
CIN 3	88	50	2.8	1.7-4.6	31	2.2	1.2-3.8	30	2.6	1.4-4.7	67	3.2	1.9-5.5

* Denotes that for women referred to colposcopy the cytological diagnosis has been substituted with the histopathological diagnosis of the colposcopy-directed biopsy, except in five cases where no histopathological diagnosis had been registered.

compared with the sexual histories of the women. HPV seropositivity was strongly related to the lifetime number of sexual partners (Table 2). HPV-16 seropositivity was more common than HPV-18 or -33 seropositivity and reached a 'plateau level' in the women with two to five lifetime partners (Table 2). None of the HPV seropositivities was significantly associated with age (not shown).

Risk of CIN in case of HPV seropositivity

Seropositivity for HPV-16, -18 or -33 collectively was associated with a significantly increased risk of the cytological diagnoses of high-grade CIN and CIN NOS (definitive CIN, but too few cells to permit grading), but not significantly associated with the low-grade cytological diagnoses (Table

Table 4. Presence of CIN 2–3 in relation to sexual history

No. of lifetime partners	No. of women	CIN 2–3							
		Positive women (%)	Crude OR	CI (95%)	Adjusted OR*	CI (95%)	Adjusted OR†	CI (95%)	
0–1	64	8	1.0	–	1.0	–	1.0	–	
2–5	223	28	4.5	1.7–15.1	3.4	1.3–9.1	2.7	0.6–15.3	
5+	164	27	4.4	1.6–15.1	3.1	1.1–10.6	1.7	0.4–9.6	

* Adjusted for HPV-16, -18, -33 antibodies.

† Adjusted for HPV DNA.

Table 5. Presence of HPV DNA in relation to sexual history

No. of lifetime partners	No. of women	HPV DNA		
		Positive women (%)	OR	CI (95%)
0–1	74	5	1.0	–
2–5	252	32	8.1	2.9–31.6
5+	186	34	9.1	3.2–36.0

3a). When comparing the HPV seropositivities to histopathological diagnoses of the biopsies that were subsequently taken, a significant two- to threefold association was found for CIN grade 3 but not for the more low-grade diagnoses, for all three HPV types (Table 3b).

Risk of CIN and HPV DNA presence depending on sexual histories

There was an approximately fourfold risk of high-grade CIN associated with having more than one lifetime partner (Table 4). The risk appeared to not increase any further with more than five lifetime partners (Table 4). A similar 'plateau effect' was seen for the risk of HPV DNA positivity which was also highly increased among women who had had more than one lifetime partner, but did not increase any further among women with more than five lifetime partners (Table 5).

Adjusting the sexual history-related risk of CIN for HPV-16/18/33 seropositivity resulted in a reduction of relative risks (Table 4). Adjusting for HPV DNA reduced the sexual history-related risk of CIN to levels that were not significantly different from unity (Table 4).

To analyse whether false-negative misclassification of HPV exposure may have affected the results of adjustment in the model, we also analysed the sexual history-related risk of CIN 2–3 in analyses restricted to HPV-16/18/33-seropositive

Table 6. Presence of CIN 2–3 in relation to sexual history among women seropositive for HPV-16, -18 and/or -33

No. of lifetime partners	No. of women	Positive women (%)	OR	CI (95%)
0–1	10	30	1.0	–
2–5	104	37	1.3	0.3–8.5
5+	92	35	1.2	0.3–8.0

women only (Table 6). There was no significant risk of CIN 2–3 associated with sexual behaviour among seropositive women. Similar analyses restricted to HPV DNA-positive women were not meaningful, because the reference category contained only a single woman.

Discussion

The within-study validation of the HPV serology for HPV-16, -18 and -33 showed a sensitivity of 65–75% for detection of type-specific infection. For HPV-16, this is in good agreement with previous studies that have mostly reported sensitivities of 50% or greater (Kirnbauer *et al.*, 1994; Chua *et al.*, 1996; de Gruijl *et al.*, 1997; Dillner *et al.*, 1996; Wideroff *et al.*, 1995; Wikström *et al.*, 1995). For HPV-18 and -33 seropositivity, the present study provides the first large-scale evaluation of sensitivity and type-specificity, which were found to be similar to HPV-16. This is of particular interest, since both HPV-18 and -33 seropositivity have been found to be associated with the risk of cervical cancer in prospective studies (Dillner *et al.*, 1997) and, in the case of HPV-18 seropositivity, also of prostate cancer (Dillner *et al.*, 1998).

The type-specificity of the serological test was evaluated by comparing the proportion of seropositive women among women positive for the other HPV DNA types with the

proportion of seropositive women among HPV-negative women. An excess of seropositive women positive for other HPV types has been documented (Dillner *et al.*, 1996; Wideroff *et al.*, 1995), but was largely explained by the sexual history of the women, suggesting that the women positive for other HPV types constitute a risk group for previously having had a type-specific infection (Dillner *et al.*, 1996). In the present study, the women positive for DNA of other HPV types had seroprevalences quite similar to those of HPV-negative women. Possibly, the comparably low mean number of sexual partners in this population may have resulted in a more limited amount of previous exposures to various HPV types. In any case, it may be concluded that the finding of increased seroprevalences among women testing DNA-positive for other HPV types is not a consistent finding in different studies using the same assay, but performed in different populations. Since the same test is used, the seropositivity to other HPV types cannot be due to cross-reactivity in the test. The likely explanation is previous exposure to type-specific HPV infection, which will be present to a variable extent in different populations.

The very high association of seroprevalences with the lifetime number of partners of the women is in accordance with a number of previous studies (Andersson-Ellström *et al.*, 1996; Viscidi *et al.*, 1997; Olsen *et al.*, 1997; Wideroff *et al.*, 1996) and further strengthens the concept that seropositivity reflects cumulative lifetime HPV exposure.

The risk of CIN was found to be elevated for HPV-16, -18 or -33 seropositivity. The HPV-16 seropositivity-associated risk was similar to what was previously found in a prospective study (Chua *et al.*, 1996), whereas there was no excess risk of CIN associated with HPV-18 or -33 seropositivity in that study. Although it is possible that the difference could be due to the fact that the present study was not a prospective cohort study, a more likely explanation is that the present study contained significantly more cases, particularly of CIN 3, and therefore was more able to detect also the modest risks associated with HPV-18 and -33 seropositivity.

That the seropositivity-associated risk was primarily found in women with CIN grade 3 lesions is in accordance with the results of non-intervention cohort studies, where HPV-16 seropositivity was associated with increased risk of progression to high-grade CIN (de Gruijl *et al.*, 1997), although there are also studies that have found HPV-16 seropositivity to be equally common among women with low-grade and high-grade CIN (Strickler *et al.*, 1997). Previous studies of subjects with new HPV infection found that transient antibody peaks that return to seronegativity after clearance of viral DNA sometimes occur (Wikström *et al.*, 1995). It is probable that very short-lived infections or virus exposures that do not result in infection might not induce a seroconversion to stable antibody levels. HPV seropositivity is also more common among women where HPV DNA has been detected on two occasions (Wideroff *et al.*, 1995; de Gruijl *et al.*, 1997).

Detection of HPV on two occasions some years apart also increases the probability that the HPV DNA test was not misclassified and increases the likelihood that the demonstration of viral DNA did indeed indicate an infection. Similarly, easily detected amounts of viral DNA ('high virus load') are more likely to reflect a correctly classified infection. Possibly, seropositivity could be used to identify the subset of HPV DNA-positive women where an infection is indeed established and risk of progression to CIN 3 exists.

In comparing the somewhat variable estimates of the sensitivity of the HPV serology, it is noteworthy that the studies that found sensitivities of 50% or greater (Kirnbauer *et al.*, 1994; Chua *et al.*, 1996; de Gruijl *et al.*, 1997; Dillner *et al.*, 1996; Wideroff *et al.*, 1995; Wikström *et al.*, 1995) were also the studies where the known attributes of HPV DNA detectability (such as dependence on recent partner change) and case-control differences between healthy women and women with CIN, were most clearly seen. As has been emphasized previously (Schiffman, 1995), in the absence of differential misclassification (which is assumed to be absent when absolutely identical samples are taken from cases and controls and sent for laboratory analysis in a blind fashion) high correlations do imply correct measurements. The present study had one of the highest ORs in the literature for risk of CIN 3 in the case of HPV DNA positivity (OR = 606) (Kjellberg *et al.*, 1998) and we therefore have reason to believe that our study has accurate measurements, resulting in accurate estimates. In studies of correlated suspected risk factors, accurate estimates are crucial since even a minor extent of misclassification will lead to erroneous conclusions due to residual confounding (Franco, 1991).

The evidence in the present study indicates that STDs other than HPV are not a risk factor for high-grade CIN, not even in determining which HPV-exposed (seropositive) women establish persistent infection. This is suggested both by the fact that there was no residual sexual behaviour-associated risk of CIN 2–3 among HPV-seropositive women and by the fact that the effect of sexual behaviour was explained by also adjusting for the presence of HPV DNA. A similar adjustment for HPV exposure of all HPV types was not possible since serological assays for all other HPV types were not available at the time of this study.

In summary, the present study found no evidence that sexual history was a risk factor for high-grade CIN, after taking HPV exposure into account. However, it should be noted that the present study had CIN 2–3 as the end-point and thus did not study the risk factors that may determine which women with CIN 2–3 develop invasive cervical cancer.

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