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## Prospective study of Human Papillomavirus and Risk of Cervical Adenocarcinoma

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### Abstract

Human papillomaviruses (HPV) are established as a major cause of cervical carcinoma. However, causality inference is dependent on prospective evidence showing that exposure predicts risk for future disease. Such evidence is available for squamous cell carcinoma, but not for cervical adenocarcinoma. We followed a population-based cohort of 994 120 women who participated in cytological screening in Sweden for a median of 6.7 years. Baseline smears from women who developed adenocarcinoma during follow-up (118 women with in situ disease and 164 with invasive disease) and their individually matched controls (1434 smears) were analyzed for HPV using PCR. Conditional logistic regression was used to estimate odds ratios (OR) of future adenocarcinoma with 95% confidence intervals (CI). Being positive for HPV 16 in the first cytologically normal smear was associated with increased risks for both future adenocarcinoma in situ (OR 11.0, 95 % CI 2.6–46.8) and invasive adenocarcinoma (OR 16.0, 95 % CI 3.8–66.7), compared to being negative for HPV 16. Similarly, an HPV 18 positive smear was associated with increased risks for adenocarcinoma in situ (OR 26.0, 95 % CI 3.5–192) and invasive adenocarcinoma (OR 28.0, 95 % CI 3.8–206), compared to an HPV 18 negative smear. Being positive for HPV 16/18 in two subsequent smears was associated with an infinite risk of both in situ and invasive adenocarcinoma. In conclusion, infections with HPV 16 and 18 are detectable up to at least 14 years before diagnosis of cervical adenocarcinoma. Our data provide prospective evidence that the association of HPV 16/18 with cervical adenocarcinoma is strong and causal.

## Keywords

Adenocarcinoma; adenocarcinoma in situ; HPV; cervical cancer; prospective

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## INTRODUCTION

Incidence rates of cervical adenocarcinoma, which accounts for 10–20 % of all cervical cancers, have increased continuously in developed countries during the last two decades, as opposed to those of squamous cell cervical carcinoma<sup>1–2</sup>. This upward trend, noted particularly among women under age 40, has occurred despite extensive cytological Pap smear screening<sup>3–6</sup>. Consequently, a deeper understanding of the etiology of cervical adenocarcinoma, and better preventive efforts are urgently called for.

A recent collaborative study indicated that the two histological forms of cervical cancer, squamous cell and adenocarcinoma, share most known risk factors<sup>7</sup>, the main one being infection with human papillomaviruses (HPV)<sup>8–9</sup>. Certain oncogenic types of HPV, in particular HPV 16 and 18, have been strongly associated with risk of cervical adenocarcinoma in several case-control studies<sup>9</sup>. Provided that early detection of HPV DNA is possible, this may offer the best means of preventing the development of adenocarcinoma, since prevention through regular cytological screening has proven to be difficult. Previous studies, however, determined HPV status only at the time of diagnosis, and therefore were unable to establish a temporal association between HPV infection and subsequent development of invasive adenocarcinoma (AC) or its precursor, adenocarcinoma in situ (AIS). To clarify the temporal association between HPV infection and the risks of in situ and invasive adenocarcinoma, we prospectively examined HPV status in repeated smears in a population-based cohort of women screened at least once for cervical cancer over a period of up to 26 years.

## METHODS

### Participants

Cytological screening with Papanicolaou (Pap) smears was gradually introduced into Sweden, starting in 1967. Since the mid-1970's, all Swedish women have been invited to screening every 3 or 4 years<sup>10</sup>. Virtually all Pap smears have been stored, and computerized records containing all information from the cytological screening are kept in the Swedish National Cervical Screening Register<sup>11</sup>. The Swedish National Cancer Registry, established in 1958, records new diagnoses of both invasive cervical cancer and severe dysplasia or cancer in situ of the cervix. The register is considered to include virtually 100% of all incident cancer cases in Sweden<sup>12</sup>.

The source population for this study comprised all Swedish women (994 120) who participated in cervical screening within eight Swedish counties sometime during the period 1969–2002. Using the same study design as in a previous investigation of cervical squamous cell carcinoma in situ<sup>13</sup>, we identified in the National Cervical Screening Register a cohort of 968 126 women, whose first registered smear during the study period was classified as cytologically normal. Records from our cohort were then linked to the National Cancer Register to identify all women with a first diagnosis of either AIS or AC after entry in our study.

We identified 121 AIS cases and 174 AC cases. Using case-control pair sampling, one woman - matched on county, date of entry into cohort (+/- 3 months), and age (+/- 1 year) - was randomly selected as an individually matched control for each AIS and AC case woman. For both members of each case-control pair, all smears taken prior to the date of diagnosis of the case were identified and retrieved from the archives. To verify the diagnoses of AIS or AC,

the histological specimens from all identified cases were reviewed by an experienced pathologist.

### Smear Analyses

Each smear was re-coded and re-labelled to ensure blinding of case-control status during DNA extraction and HPV analysis. Samples belonging to the same case-control pair were included in the same analysis batch. After DNA extraction by methods described elsewhere<sup>14</sup>, all smears were analysed for the presence of seven low-risk HPV types (HPV 6, 7, 11, 42, 43, 70, and 90), and 16 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82). We used polymerase chain reaction (PCR) amplification of a consensus region using GP5+/6+ primers<sup>15</sup>, followed by detection of biotinylated HPV amplicons by hybridisation to short oligonucleotide probes covalently linked to fluorescence-labeled carboxy-coated polystyrene beads on the Bioplex 200 Luminex system (Biorad, CA, USA), as described previously<sup>16</sup>. The amount of amplifiable DNA in the samples was determined by real-time PCR for the housekeeping  $\beta$ -globin gene.

Initially, 1622 smears were identified. We subsequently excluded 113 smears with missing or negative  $\beta$ -globin value, 28 because they were from incomplete case-control pairs, and 47 smears because they were obtained within the year immediately preceding diagnosis as part of diagnostic work-up of the case. Consequently, one AIS case, five AC cases, and seven control women were excluded since they did not have any eligible Pap smears. Another two AIS cases and five AC cases were excluded because the case-control pairs were incomplete or because smears were taken on the same day as diagnosis of the disease. Finally, 1434 smears from 282 (118 AIS and 164 AC) complete case-control pairs, in which each woman had at least one  $\beta$ -globin-positive smear, remained for the statistical analyses.

### Statistical Analysis

Due to the matched case-control design, conditional logistic regression was used to estimate odds ratios (ORs). Analyses were conditioned on the case-control pair variable that indicates a matched case-control pair. Cervical cancer being a rare disease, we interpreted the ORs as estimates of relative risk (RR), namely the risk of an outcome (AIS or AC) in women exposed to a specific HPV strain, relative to that in women not exposed to that strain. All low risk HPV types (LRHPV) and high risk HPV types excluding HPV 16 and 18 (non-16/18 HRHPV) were analyzed as combined categories. Pooled risk estimates for these HPV groupings were calculated, as we lacked statistical power to obtain reliable HPV type-specific risk estimates for single types, except for HPV 16 and 18. We also lacked power to assess multiple HPV infections separately. Separate analyses were conducted to assess HPV presence in the first and in the last smear prior to diagnosis for the case. Exposure categories were defined as follows; a) HPV 16 – first/last smear positive for HPV 16; b) HPV 18 – first/last smear positive for HPV 18; c) HPV 16/18 – first/last smear positive for HPV 16 and/or HPV 18; d) non-16/18 HRHPV – first/last smear positive for one or more high risk HPV types other than HPV 16 or HPV 18; e) LRHPV – first/last smear positive for one or more low risk HPV types. Attributable risk proportions were calculated based on ORs obtained from the conditional logistic regression models<sup>17</sup>. HPV prevalences among cases and controls were estimated by dividing time prior to diagnosis into seven intervals (two-year intervals up to 14 years prior and one open interval prior to that) and calculating the proportion of HPV-positive smears in each interval; patients could contribute smears to several intervals, however, results of smears from the same patient falling into the same interval were averaged. Prevalences were plotted over median time before diagnosis in each interval, and a loess smoother weighted with the number of observations per interval was added. For studying the effect of persistent HPV16/18 infection on adenocarcinoma risk, we identified all subjects in case-control pairs where both cases and controls had at least two smears prior to diagnosis. Based on the HPV test results of the first

two consecutive smears, subjects were classified as either double positive, double negative or mixed, i.e. positive/negative or negative/positive. Data are presented in contingency tables. ORs for exposure categories were estimated with conditional logistic regression. All calculations were done using R version 2.8.1 18.

Informed consent from participating women was not required according to the Karolinska Institute Ethics Review Board which approved the study.

## RESULTS

### Characteristics of the Participants

The characteristics of the 118 AIS and 164 AC cases and their matched control women are described in Table 1. The AIS cases were younger at time of diagnosis (median age 36, range 20–60 years) compared to the AC cases (median age 43, range 24–88 years). The AIS cases and controls were followed for a median time of about 7 years (range 1–26 years), and the AC cases had a median follow-up time of around 6 years (range 1–21 years). The number of smears registered during follow-up were fairly similar for AIS and AC cases and their controls, with a median of 2 to 3 per woman and a range from 1 to 13 (Table 1).

### HPV Prevalence

In the first smear at study entry, we detected one or more high risk HPV types in 45% of the AIS cases and 8% of the controls, while the corresponding figures were 40% for AC cases and 10% for controls (Table 2). The two most common types detected in the first smear of cases were HPV 16 and HPV 18. Multiple high risk types were detected in 13% of AIS cases and in 9% of AC cases, but in only 1% of the control women. Table 2 also shows the HPV prevalence in the last available smear preceding diagnosis of AIS or AC. HPV 16 and 18 prevalence increased from first to last smear in both AIS and AC cases, whereas the prevalence among controls remained fairly stable (Table 2).

Among AIS cases, the probability of being positive for HPV 16 or 18 increased as time to diagnosis decreased, from about 35% 14 years or more prior to diagnosis to about 57% just before diagnosis (Figure 1A). For AC cases, the probability of HPV 16 or 18 positivity was 20% 14 years or more before diagnosis, and increased to 47% close to diagnosis (Figure 1B). The probability of non-HPV 16/18 high-risk HPV infection in AIS and AC cases was below 15% at the start of follow-up, and did not change appreciably as time of diagnosis approached (Figure 1C and D). Among controls, the prevalence remained stable below 10% throughout the follow-up period (Figure 1–2 A and B). The probability of being infected with low-risk HPV was similar for AIS and AC cases and remained constant over time for both cases and controls (Figure 1E and F).

### Risk Associations

Being positive for HPV in the first cytologically normal smear was associated with almost 11-fold (95% CI 2.6–46.8) increased risk of AIS, while HPV 18 positivity increased the risk 26-fold (95% CI 3.5–192), compared to being negative for the corresponding HPV strain in the first smear (Table 3). The risk association increased from first to last smear for both HPV 16 and 18 (Table 3). The risk of developing AC in relation to infection with HPV 16 or 18 in the first smear was somewhat higher than for AIS (OR 16.0, 95% CI 3.8–66.7 for HPV 16, and OR 28.0, 95% CI 3.8–206 for HPV 18), but the change in risk estimates from first to last smear was less pronounced than for AIS (Table 3). Infections with other high-risk HPV types were not associated with any statistically significant increase in risk of AIS or AC (tables 3 and 4).

In the studied cohort, the HPV 16 and/or 18 attributable risk proportion (ARP) in the first smear was 36% (95% CI 26–45%) for AIS and 35% (95% CI 26–41%) for AC. The corresponding HPV 16/18 ARP for the last smear prior to diagnosis was 52% (95% CI 43–62%) for AIS and 43% (95% CI 35–51%) for AC.

We identified at least two eligible smears for 75 case-control pairs with AIS and 55 for AC. The average time interval between the smears was 3.0 years for AIS (sd=2.0 years), and 3.1 years for AC (sd=2.3 years), with no significant differences between cases and controls ( $p=0.44$  and  $p=0.82$ , respectively). Being positive for HPV 16/18 in the first smear and negative in the second smear conferred ORs 6.0 (95% CI 1.3–26.8) and 2.9 (95% CI 0.1–84.1) for AIS and AC, respectively, compared to being negative in both smears (Table 5). Being negative for HPV 16/18 in the first smear and positive in the second smear conferred OR 7.5 (95% CI 1.7–32.8) and 8.4 (95% CI 0.9–79.7) for AIS and AC, respectively, while all double positive subjects were cases for both forms of the disease ( $p=4 \times 10^{-7}$  for AIS and  $p=1 \times 10^{-6}$  for AC, both corresponding to  $OR=\infty$  in conditional logistic regression)(Table 5). We found similar results for exposure to HPV 16 and HPV 18 on their own (data not shown), however, no other strain or combination of strains showed significance, unless HPV 16/18 were included (data not shown).

## DISCUSSION

In this large prospective study, we found that infection with HPV 16 and 18, detected in cytologically normal smears up to at least 14 years before diagnosis, were associated with substantially increased risks of subsequent development of invasive adenocarcinoma (AC) and its precursor, adenocarcinoma in situ (AIS). The risk estimates were generally higher for HPV 18, particularly when they were based on the first eligible smear. Interestingly, the type-specific risk associations did not differ substantially between the two diagnoses, AIS and AC. Double positivity in two subsequent smears conferred an infinite risk increase for both AIS and AC.

We determined HPV status in archival smears, taken repeatedly up to 26 years before the diagnosis of AIS and AC, in cases and control women participating in cytological screening in Sweden. Because of the nested design, our study preserved the validity of the underlying cohort, and our risk estimates are likely to reflect the true risk for the cohort under study. Previous studies have been case series<sup>19–22</sup> where no risk association could be assessed, or case-control studies<sup>7, 9, 23–24</sup>, where the temporality of the association could not be addressed, because HPV status was assessed concurrently with disease diagnosis. Because we assessed exposure to HPV up to 26 years before the date of diagnosis of the case (Table 1), our study provides strong evidence in support of the notion that infection with HPV precedes the development of adenocarcinoma of the cervix. Involvement of a substantial proportion of the eligible population in the screening program enhances the generalizability of our findings. If there were more than one smear taken prior to one year before diagnosis we only included the first, since case women had more smears registered during the last year before diagnosis (92 versus 39 smears for control women), probably collected as part of the diagnostic work-up.

One possible limitation of our study is the lack of data on confounding or effect-modifying factors such as oral contraceptive use, smoking, parity, and other sexual transmitted infections<sup>25</sup>. However, given the magnitude of the associations with HPV, it is unlikely that adjustment for other factors, themselves modestly associated with risk<sup>7, 9</sup>, would have changed our conclusions that HPV 16 and 18 are strongly associated with adenocarcinoma of the cervix. Another possible limitation is the use of archival Pap smears which might lower the sensitivity of our HPV analyses and thus underestimate the true HPV prevalence in our cohort. Most likely, this underestimation would be non-differential for cases and controls, driving the

estimated odds ratios towards the null, and also entailing an underestimation of the true attributable risk proportions.

The overall HPV 16/18 prevalence among prospective case women increased as time to diagnosis decreased, reaching a level of about 57% one year before diagnosis (Figure 1A). This estimate is somewhat lower than what has been reported in previous studies<sup>7, 9</sup>. Again, this could be due to our estimates being based on archival smears. We observed that the HPV type-specific risks for AIS and AC are not the same as those for squamous cell carcinoma; in contrast to squamous cell carcinoma, HPV18 had a stronger association with AIS and AC than HPV 16, and other HPV types classified as oncogenic for squamous cell cancer were not statistically significantly associated with risk of AIS or AC (tables 3 and 4). These findings are in accordance with several previous studies that have consistently found HPV 18 to be preferentially associated with adenocarcinoma rather than with squamous cell carcinoma of the cervix<sup>7</sup>. In contrast to the results of our study, Castellsagué et al. reported an association with high risk types other than HPV 16 and 18 with cervical adenocarcinoma<sup>9</sup>. However, these contradictory results should be interpreted cautiously, since both our and their study suffered from low statistical power to investigate associations with HPV types of low prevalence.

The strong link between infection with HPV 16 or 18 and malignant transformation of the glandular epithelium of the cervix suggests that screening for these HPV types might be a useful tool for improving the prevention and/or early detection of adenocarcinomas of the cervix, which has proven to be difficult through regular cytological screening<sup>26</sup>. It was recently estimated that Swedish women who did not attend to regular cytological screening had a modest 60% increased risk of being diagnosed with cervical adenocarcinoma but a 200% increased risk of squamous cell carcinoma, in comparison to women who took part in the screening<sup>27</sup>. Hence, cytological screening entails substantially lower protection against adenocarcinoma than squamous cell cancer of the cervix. Our results point to a strong temporal association between HPV and cervical adenocarcinoma, which has previously not been possible to demonstrate. Detection of HPV 16 and 18 as objective molecular markers of infection could allow diagnosis of cervical adenocarcinomas at an earlier stage in the natural history of the disease. In particular, women positive for HPV 16/18 on two subsequent tests seem to be at very high risk of acquiring the disease. Furthermore, HPV analyses of precursor lesions, namely adenocarcinoma in situ and glandular dysplasia, may help to find women at risk of further progression to invasive disease, and thus improve the prevention of adenocarcinomas.

We conservatively estimate that 35–52% of all adenocarcinoma cases in our cohort are attributable to infection with HPV 16 or 18. Our results also indicate that the first generation of HPV vaccines, targeting HPV 16 and 18 infections, might prevent at least one third of cervical adenocarcinomas and its precursor lesions in our cohort. Despite the worrying increase in adenocarcinoma rates in the last decades, there are reasons for optimism. Through a combination of continuous public education about transmission of HPV, improved detection of precursor lesions of cervical carcinoma by incorporating HPV testing into the screening programs, and by vaccination against HPV 16 and 18, carcinoma of the cervix may become one of the major forms of cancer that is most preventable on a global scale.

We conclude that infections with HPV 16 and 18, detected at least 14 years prior to diagnosis while no cytological changes are yet evident, are associated with increased risk of developing both in situ and invasive cervical adenocarcinoma. At least one-third of the incident cases of cervical adenocarcinoma should be preventable through the currently available vaccines against HPV.

### Novelty and Impact

This study provides prospective evidence supporting that the strong association of HPV16/18 with cervical adenocarcinoma is indeed of etiological significance. Availability of such evidence is essential for efforts aimed at preventing cervical adenocarcinoma by way of preventing HPV infections. Furthermore, the fact that HPV positivity strongly predicted development of cervical adenocarcinoma also many years into the future further implies that HPV-based cervical screening programs could have a lasting protective effect against this disease.

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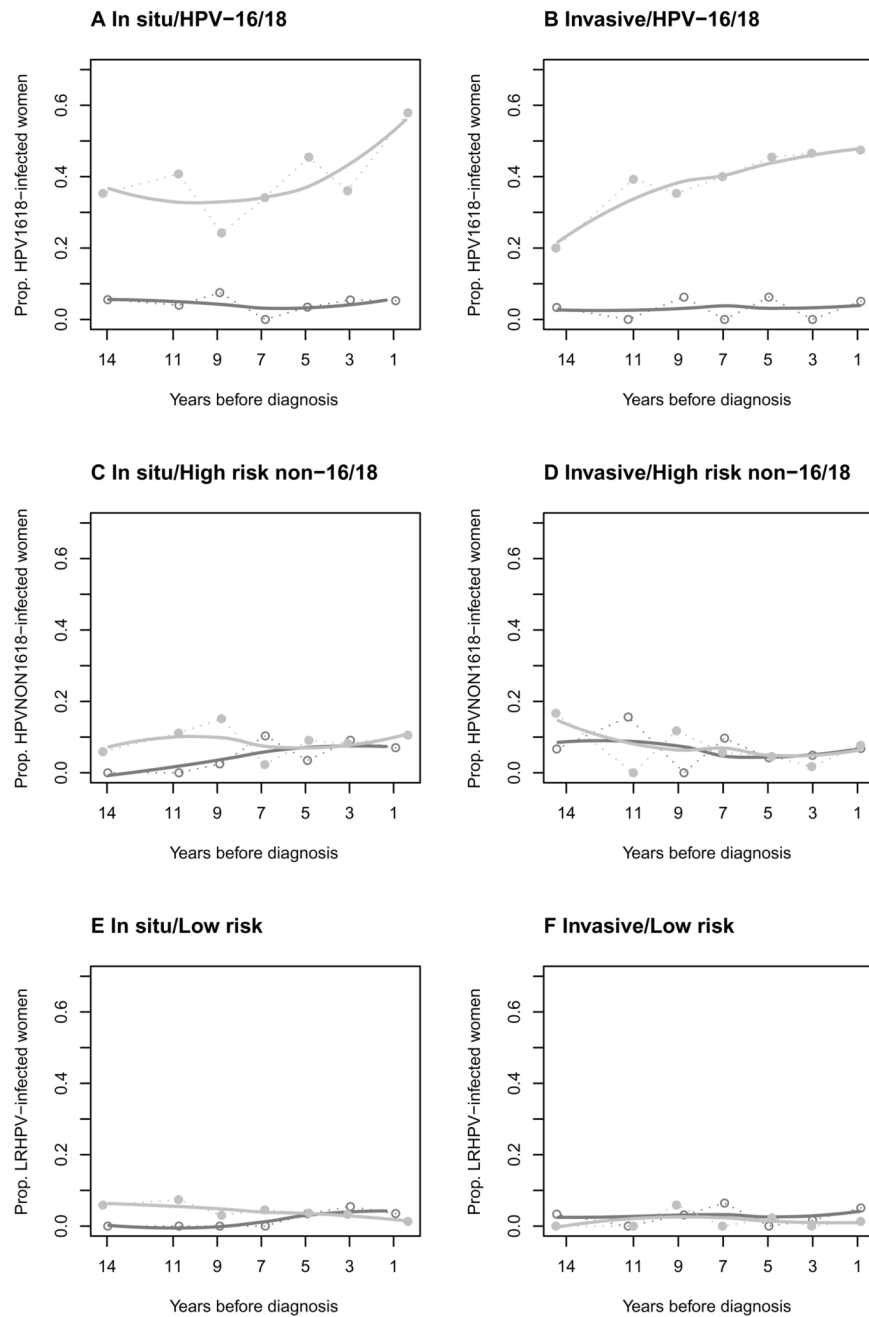
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**Figure 1.** A–F. Prevalences of different HPV strains for cases and controls over several time periods prior to diagnosis/end of follow-up. See Methods for details. The grey line represents cases and the black line controls.

**Table 1**

Characteristics of the participants.

	Adenocarcinoma in situ		Invasive adenocarcinoma	
	Cases	Controls	Cases	Controls
<b>Number of study subjects</b>	118	118	164	164
<b>Age at diagnosis<sup>1</sup></b>	36 (20 – 60)	36 (19 – 61)	43 (24 – 88)	43 (24 – 88)
<b>Age at entry</b>	27 (16 – 57)	28 (16 – 57)	36.5 (17 – 82)	36 (17 – 81)
<b>Study time</b>	7.3 (1 – 26)	7.45 (1 – 25.9)	6.1 (1 – 21.1)	5.9 (1 – 21.3)
<b>Number of smears</b>	373	324	392	345
<b>Median no. of smears per subject</b>	3 (1 – 13)	2 (1 – 12)	2 (1 – 9)	2 (1 – 8)

<sup>1</sup>Median age and study time in years (range).

**Table 2**

Prevalence of specific HPV types and HPV type groupings in first and last Pap smear for adenocarcinoma in situ, and invasive adenocarcinoma cases and their controls

HPV Type	Low Risk (LR) or High Risk (HR)	Adenocarcinoma in situ				Invasive adenocarcinoma			
		First smear		Last smear		First smear		Last smear	
		Cases N=118 n (%)	Controls N=118 n (%)	Cases N=118 n (%)	Controls N=118 n (%)	Cases N=164 n (%)	Controls N=164 n (%)	Cases N=164 n (%)	Controls N=164 n (%)
HPV 6	LR	0	0	0	0	0	0	0	1 (1)
HPV 7	LR	0	0	0	0	0	0	0	0
HPV 11	LR	0	0	0	1 (1)	0	2 (1)	1 (1)	1 (1)
HPV 16	HR	23 (19)	3 (3)	35 (30)	5 (4)	33 (20)	3 (2)	38 (23)	2 (1)
HPV 18	HR	26 (22)	1 (1)	34 (29)	0	28 (17)	1 (1)	38 (23)	2 (1)
HPV 31	HR	3 (3)	0	4 (3)	3 (3)	5 (3)	4 (2)	5 (3)	3 (2)
HPV 33	HR	1 (1)	0	2 (2)	0	3 (2)	0	4 (2)	0
HPV 35	HR	1 (1)	1 (1)	1 (1)	0	1 (1)	1 (1)	1 (1)	0
HPV 39	HR	0	1 (1)	3 (3)	0	0	0	0	0
HPV 42	LR	3 (3)	3 (3)	1 (3)	3 (3)	0	1 (1)	0	0
HPV 43	LR	1 (1)	0	1 (1)	0	0	1 (1)	1 (1)	1 (1)
HPV 45	HR	4 (3)	1 (1)	4 (3)	1 (1)	5 (3)	2 (1)	8 (5)	1 (1)
HPV 51	HR	3 (3)	0	2 (2)	0	2 (1)	0	3 (2)	0
HPV 52	HR	1 (1)	0	2 (2)	1 (1)	1 (1)	0	1 (1)	1 (1)
HPV 56	HR	0	1 (1)	2 (2)	0	1 (1)	0	2 (1)	1 (1)
HPV 58	HR	2 (2)	0	1 (1)	0	0	2 (1)	0	1 (1)
HPV 59	HR	2 (2)	0	1 (1)	0	1 (1)	2 (1)	3 (2)	1 (1)
HPV 66	HR	4 (3)	0	2 (2)	1 (1)	5 (3)	0	1 (1)	1 (1)
HPV 68	HR	1 (1)	0	1 (1)	0	0	0	0	0
HPV 70	LR	0	0	0	0	0	1 (1)	2 (2)	2 (1)
HPV 73	HR	0	0	0	0	1 (1)	0	0	0
HPV 82	HR	0	0	0	0	0	0	0	0
HPV 16/18	HR	45 (38)	4 (3)	63 (53)	5 (4)	58 (35)	4 (2)	73 (45)	4 (2)

HPV Type	Low Risk (LR) or High Risk (HR)	Adenocarcinoma in situ				Invasive adenocarcinoma			
		First smear		Last smear		First smear		Last smear	
		Cases N=118 n (%)	Controls N=118 n (%)	Cases N=118 n (%)	Controls N=118 n (%)	Cases N=164 n (%)	Controls N=164 n (%)	Cases N=164 n (%)	Controls N=164 n (%)
Non-16/18	HR	8 (7)	3 (3)	11 (9)	4 (3)	7 (4)	9 (5)	12 (7)	7 (4)
Any LRHPV	LR	4 (3)	3 (3)	2 (2)	4 (3)	0	5 (3)	2 (1)	5 (3)
Any HRHPV	HR	53 (45)	7 (6)	74 (63)	9 (8)	65 (40)	13 (8)	85 (52)	11 (7)
Any HPV	HR or LR	53 (45)	10 (8)	75 (64)	12 (10)	65 (40)	17 (10)	85 (52)	15 (9)
Multiple HPV types	HR or LR	15 (13)	1 (1)	16 (14)	2 (2)	15 (9)	2 (1)	17 (10)	3 (2)

**Table 3**

Conditional logistic regression showing odds ratios (OR) and 95% confidence interval (CI) of in situ adenocarcinoma among women with HPV positive first and last smear.

	Adenocarcinoma in situ					
	First smear			Last smear		
	Positive cases/controls	OR	95% CI	Positive cases/controls	OR	95% CI
<b>HPV 16</b>	23/3	11.0	2.6–46.8	35/5	32.0	4.2–227
<b>HPV 18</b>	26/1	26.0	3.5–192	34/0	∞	0–∞
<b>HPV 16/18</b>	45/4	14.7	4.5–47.2	63/5	59.0	8.2–426
<b>Non-16/18 HRHPV</b>	8/3	3.5	0.7–16.8	11/4	2.8	0.9–8.6
<b>LRHPV</b>	4/3	1.3	0.3–6.0	2/4	0.5	0.1–2.7

\* Perfect separation, no estimate

HRHPV denotes High Risk HPV and LRHPV Low Risk HPV.

Conditional logistic regression showing odds ratios (OR) and 95% confidence interval (CI) of invasive adenocarcinoma among women with HPV positive first and last smear.

**Table 4**

	Invasive adenocarcinoma					
	First smear			Last smear		
	Positive cases/controls	OR	95% CI	Positive cases/controls	OR	95% CI
<b>HPV 16</b>	33/3	16.0	3.8–66.7	38/2	19.0	4.6–78.8
<b>HPV 18</b>	28/1	28.0	3.8–206	38/2	19.0	4.6–78.8
<b>HPV 16/18</b>	58/4	19.0	6.0–60.7	73/4	24.0	7.6–76.2
<b>Non-16/18 HRHPV</b>	7/9	0.8	0.3–2.1	12/7	1.7	0.7–4.4
<b>LRHPV</b>	0/5	0.8	0.3–2.1	2/5	0.4	0.1–2.1

HRHPV denotes High Risk HPV and LRHPV Low Risk HPV.

**Table 5**  
HPV16/18 infection status for subjects in case-control pairs with at least two smears

HPV16/18 in first two smears	Adenocarcinoma in situ				Invasive adenocarcinoma					
	Cases (%)	Controls (%)	OR	95% CI <sup>a</sup>	p-value <sup>b</sup>	Cases (%)	Controls (%)	OR	95% CI <sup>a</sup>	p-value <sup>b</sup>
--	31 (41%)	70 (93%)	1.0	-	-	30 (54%)	52 (94%)	1.0	-	-
+–	12 (16%)	3 (4%)	6.0	1.3–26.8	5x10 <sup>-3</sup>	1 (2%)	2 (4%)	2.9	0.1–84.0	5x10 <sup>-1</sup>
-+	15 (20%)	2 (3%)	7.5	1.7–32.8	8x10 <sup>-4</sup>	7 (13%)	1 (2%)	8.4	0.9–79.7	2x10 <sup>-2</sup>
++	17 (23%)	0 (0%)	∞	-	4x10 <sup>-7</sup>	17 (31%)	0 (0%)	∞	-	1x10 <sup>-6</sup>
Total	75 (100%)	75 (100%)				55 (100%)	55 (100%)			

<sup>a</sup> based on Wald-statistic

<sup>b</sup> based on likelihood ratio test