Prospective Study of Human Papillomavirus (HPV) Types, HPV Persistence, and Risk of Squamous Cell Carcinoma of the Cervix

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Abstract

Background: The link between squamous cell cervical carcinoma and human papillomavirus (HPV) 16/18 is well established, but the magnitude of the risk association is uncertain and the importance of other high-risk HPV (HRHPV) types is unclear.

Methods: In two prospective nested case-control series among women participating in cytologic screening in Sweden, we collected 2,772 cervical smears from 515 women with cancer *in situ* (CIS), 315 with invasive squamous cell carcinoma (SCC), and individually matched controls. All smears were tested for HPV with PCR assays, and the median follow-up until diagnosis was 5 to 7 years. Conditional logistic regression was used to estimate relative risks (RR) and 95% confidence intervals (95% CI).

Results: The presence of HPV16/18 in the first smear was associated with 8.5-fold (95% CI, 5.3-13.7) and 18.6-fold (95% CI, 9.0-38.9) increased risks of CIS and SCC, respectively, compared with women negative for HPV. Infection with other HRHPV types in the first smear was also associated with significantly increased risks for both CIS and SCC. Persistence of HPV16 infection conferred a RR of 18.5 (95% CI, 6.5-52.9) for CIS and 19.5 (95% CI, 4.7-81.7) for SCC. The HPV16/18 attributable risk proportion was estimated at 30% to 50% for CIS, and 41% to 47% for SCC. Other HRHPV types also conferred significant proportions.

Conclusions: Our large population-based study provides quantification of risks for different HPV types and prospective evidence that non-16/18 HRHPV types increase the risk for future cervical cancer.

Impact: This study gives further insights into cervical cancer risk stratification with implications for HPV-based prevention strategies. *Cancer Epidemiol Biomarkers Prev; 19(10); 2469–78. ©2010 AACR.*

Introduction

With new vaccines against the two most oncogenic human papillomavirus (HPV) types (HPV16 and HPV18), the future incidence of cervical cancer is likely to radically decrease. Randomized trials have shown virtually complete protection against HPV16- and HPV18-related cervical intraepithelial neoplasia, grade 2 or worse (CIN2+), following prophylactic HPV vaccination (1-3). Nevertheless, it will take decades for vaccination to take effect on cervical cancer rates, and the need to improve screening practices continues. Uncertainties also exist regarding the future dynamics of non-16/18 high-risk HPV (HRHPV) types following vaccine deployment, including the degree and duration of cross-protection of the current vaccines against these other types.

For women currently undergoing cervical screening, HPV-based screening has emerged as the way to improve prevention (4, 5). Hence, close investigation of the future cancer risks associated with HPV infection of different types, which are among the most common sexually transmitted infections in the world, is needed to develop sensible tools for risk stratification. To this end, we prospectively examined the risks of developing *in situ* and invasive squamous cell cervical carcinoma associated with different HPV types and with persistent HPV16

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infection, in a large population-based cohort of Swedish women. Our results may carry implications for future HPV-based screening programs, and continued development of HPV vaccines.

Materials and Methods

Participants

Starting in 1967, cytologic screening with Papanicolaou (Pap) smears was gradually introduced in Sweden. Since the mid-1970s, all Swedish women have been invited for screening every three or four years (6). Virtually all smears have been stored, and records containing all information from the cytologic screening are computerized in the Swedish National Cervical Screening Register (NCSR; ref. 7). The Swedish National Cancer Registry (NCR), established in 1958, records all new diagnoses of both cancer *in situ* and invasive cervical cancer. The register is considered to be virtually 100% complete (8).

The source population for this study comprised all Swedish women (757,690) who participated in cervical screening within at least one of seven Swedish counties some time during the period 1969-2002. Using the NCSR, we identified a cohort of 739,072 women whose first registered smear during the study period was classified as cytologically normal (PAP = 1). Records from our cohort were then linked to the NCR to identify a random sample of women with a first diagnosis of cancer *in situ* (CIS) and all women with a first diagnosis of invasive squamous cell carcinoma (SCC) after entry in our study. A diagnosis of CIS in the Swedish NCR translates to a diagnosis of CIN, grade 3 (CIN3).

We identified 515 CIS cases and 315 SCC cases. Using case-control sampling, one woman, matched on county, date of entry into cohort (±3 months), and age at first normal smear (±1 year), was randomly selected as an individually matched control for each CIS and SCC case. The smears taken prior to the date of diagnosis of the case in each case-control pair were identified and requested from the archives. To verify the diagnoses of

CIS or SCC, histologic specimens from the identified cases were reviewed by our pathologist (AL).

Smear analyses

Each smear was recoded and relabeled 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. DNA extraction was done by validated methods described elsewhere (9). The risk of cross-contamination of archival smears was evaluated and found to be low (9). All smears were analyzed for the presence of seven low-risk HPV (LRHPV) types (HPV 6, 7, 11, 42, 43, 70, and 90) and 16 HRHPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82). The PCR amplification of a consensus region using GP5+/6+ primers (10), was followed by detection of biotinylated HPV amplicons by hybridization to short oligonucleotide probes covalently linked to fluorescence-labeled carboxy-coated polystyrene beads on the Bioplex 200 Luminex system (Biorad), as described previously (11). Positive controls (HPV16 DNA) and multiple negative controls (Sigma water) were included in all runs to ensure absence of contamination. The amount of amplifiable DNA in the samples was determined by real-time PCR for the housekeeping β-globin gene. All laboratory analyses were done with the analyzing laboratory (the WHO HPV LabNet Global Reference Laboratory, Malmö, Sweden) being blinded to information about the samples.

Initially, 4,526 smears were retrieved from the archives. We subsequently excluded 102 smears with negative β-globin value and 18 with inconclusive HPV status. Furthermore, we excluded 50 smears from 23 incomplete case-control pairs because either the case or the control did not have any eligible Pap smears, and 20 smears that were taken on the day of the diagnosis of the case.

For the analysis of risk associations with different HPV types, we present results from the first and last smear, comprising a total of 2,772 smears from 830 (515 CIS and 315 SCC) complete case-control pairs. The persistence of HPV16 infection was analyzed using the

Table 1. Characteristics of the participants

	Cancer *in situ*		Invasive squamous cell carcinoma	
	Cases	Controls	Cases	Controls
Number of study subjects	515	515	315	315
Age at diagnosis, y*	33 (18-64)	33 (18-63)	40 (24-81)	40 (24-81)
Age at entry, y	26 (15-60)	26 (15-60)	32 (15-78)	32 (15-75)
Study time, y				
Time from first smear to diagnosis	5.3 (0.03-17.5)	5.4 (0.01-17.3)	7.2 (0.01-17.8)	7.6 (0.06-17.8)
Time from last smear to diagnosis	0.6 (0.01-16.2)	2.4 (0.01-16.2)	1.8 (0.01-15.9)	2.8 (0.01-14.7)
Median no. of smears per subject (range)	2 (1-13)	2 (1-11)	2 (1-15)	2 (1-11)

*For controls age at time of diagnosis of their respective case.

Table 2. Prevalence of specific HPV types and HPV type groupings in first and last Pap smear for cancer *in situ* and invasive squamous cell carcinoma cases and their matched controls

HPV type	High-risk (HR) or Low-risk (LR)	Cancer *in situ*				Invasive squamous cell carcinoma			
		First smear		Last smear		First smear		Last smear	
		Cases (n = 515), No. (%)*	Controls (n = 515), No. (%)*	Cases (n = 515), No. (%)*	Controls (n = 515), No. (%)*	Cases (n = 315), No. (%)*	Controls (n = 315), No. (%)*	Cases (n = 315), No. (%)*	Controls (n = 315), No. (%)*
Any HPV	HR/LR	301 (59)	94 (18)	407 (79)	72 (14)	191 (61)	58 (18)	224 (71)	45 (14)
HPV-16/18	HR	179 (35)	34 (7)	262 (51)	26 (5)	140 (44)	21 (7)	158 (50)	17 (5)
Non-16/18 HRHPV	HR	146 (28)	54 (11)	198 (39)	35 (7)	65 (21)	35 (11)	77 (24)	20 (6)
Any HRHPV	HR	288 (56)	82 (16)	396 (78)	57 (11)	184 (58)	49 (16)	220 (70)	33 (11)
Any LRHPV	LR	34 (7)	19 (4)	32 (6)	16 (3)	12 (4)	13 (4)	8 (3)	12 (4)
Multiple HPV types	HR/LR	57 (11)	14 (3)	82 (16)	9 (2)	28 (9)	11 (4)	19 (6)	6 (2)
HPV-6	LR	7 (1)	2 (0)	4 (0)	3 (1)	1 (0)	0	0	2 (1)
HPV-7	LR	0	0	1 (0)	1 (0)	0	0	0	0
HPV-11	LR	5 (1)	1 (0)	4 (0)	1 (0)	0	1 (0)	0	1 (0)
HPV-16	HR	158 (31)	28 (5)	235 (46)	19 (4)	118 (38)	16 (5)	131 (42)	12 (4)
HPV-18	HR	30 (6)	7 (1)	36 (7)	8 (2)	27 (9)	6 (2)	29 (9)	6 (2)
HPV-31	HR	34 (7)	17 (3)	49 (10)	8 (2)	13 (4)	7 (2)	16 (5)	3 (1)
HPV-33	HR	34 (7)	5 (1)	41 (8)	4 (1)	16 (5)	2 (1)	16 (5)	2 (1)
HPV-35	HR	11 (2)	3 (1)	14 (3)	2 (0)	2 (1)	4 (1)	3 (1)	0
HPV-39	HR	5 (1)	3 (1)	7 (1)	4 (1)	4 (1)	1 (0)	5 (2)	1 (0)
HPV-42	LR	16 (3)	12 (2)	20 (4)	8 (2)	7 (2)	9 (3)	6 (2)	7 (2)
HPV-43	LR	3 (1)	2 (0)	1 (0)	1 (0)	1 (0)	2 (1)	1 (0)	1 (0)
HPV-45	HR	16 (3)	3 (1)	24 (5)	4 (1)	15 (5)	6 (2)	25 (8)	7 (2)
HPV-51	HR	8 (2)	5 (1)	14 (3)	3 (1)	3 (1)	2 (1)	1 (0)	1 (0)
HPV-52	HR	18 (4)	4 (1)	24 (5)	5 (1)	3 (1)	4 (1)	4 (1)	1 (0)
HPV-56	HR	14 (3)	9 (2)	19 (4)	8 (2)	5 (2)	2 (1)	9 (3)	2 (1)
HPV-58	HR	10 (2)	3 (1)	15 (3)	3 (1)	1 (0)	2 (1)	1 (0)	2 (1)
HPV-59	HR	26 (5)	10 (2)	33 (6)	7 (1)	15 (5)	9 (3)	13 (4)	6 (2)
HPV-66	HR	10 (2)	5 (1)	13 (3)	3 (1)	5 (2)	5 (2)	3 (1)	4 (1)
HPV-68	HR	1 (0)	1 (0)	2 (0)	1 (0)	1 (0)	1 (0)	2 (1)	0
HPV-70	LR	4 (1)	3 (1)	2 (0)	4 (1)	3 (1)	2 (1)	1 (0)	2 (1)
HPV-73	HR	0	1 (0)	0	2 (0)	1 (0)	2 (1)	0	1 (0)
HPV-82	HR	1 (0)	0	3 (1)	0	0	0	0	0

*Addition of individual HPV types exceeds the number of women due to multiple HPV infections in some women. Therefore, the sum of percentages of positive HPV infections may exceed 100%.
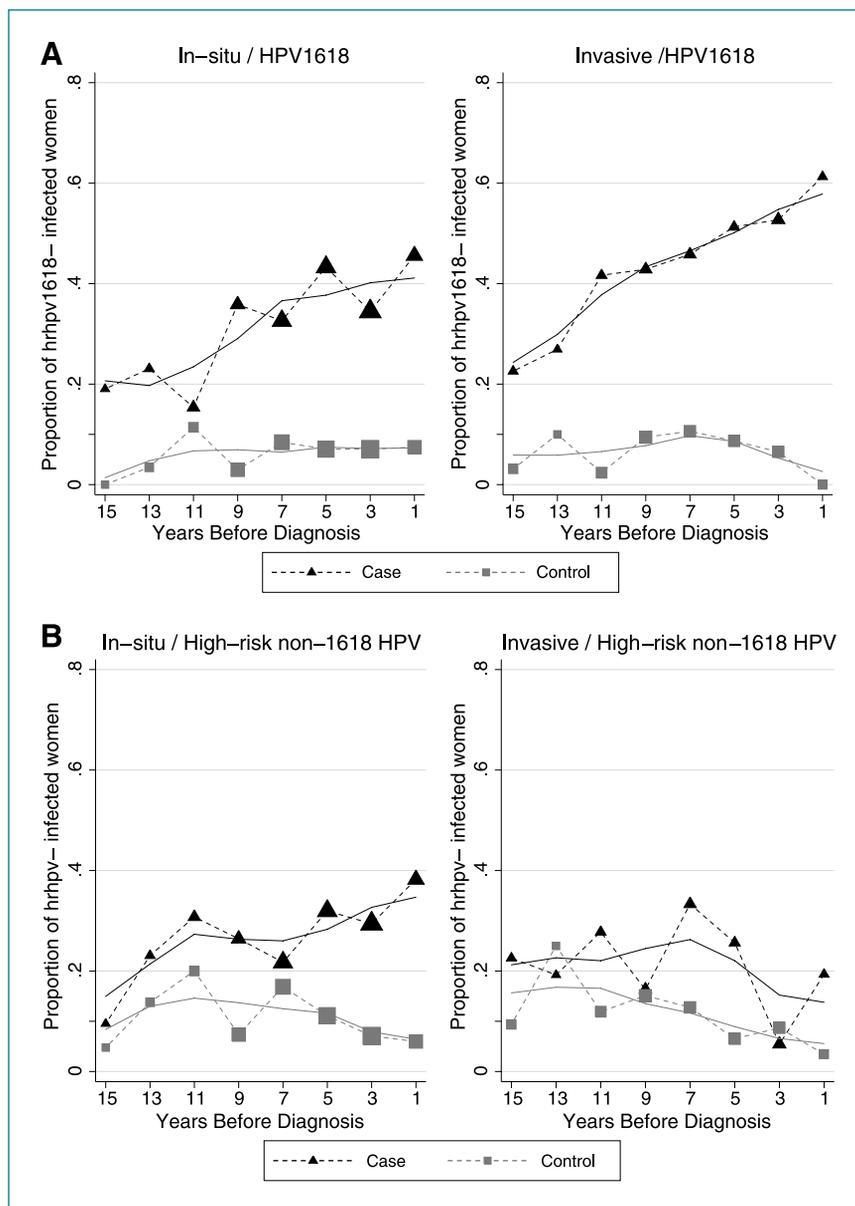
Figure 1. A to C, proportion of HPV-infected (first smear only) women by cancer type, HPV subtype, and case/control status (the size of the scatters is proportional to the number of women that were included in the calculations).

first two consecutive smears (hence women with only one smear were excluded). Cases with both smears taken within the year of their diagnosis were excluded (only applicable to one case-control pair). Using these criteria, 1,584 smears from 396 (241 CIS and 155 SCC) complete case-control pairs remained for the persistence analysis.

Statistical analyses

Due to the matched design, conditional logistic regression was used to estimate odds ratios (OR). These were interpreted as estimates of relative risk (RR) of CIS or SCC in HPV-exposed women, relative to women unexposed to the HPV strain(s) under investigation and

relative to women unexposed to any HPV. Pooled risk estimates were calculated for all LRHPV types and for all HRHPV types excluding HPV16 and 18 (non-16/18 HRHPV). We analyzed separately the risk associated with HPV presence in the first and in the last smear prior to the diagnosis of the case.

We defined exposure categories as follows: (*a*) HPV16, the first/last smear being positive for HPV16; (*b*) HPV18, the first/last smear being positive for HPV18; (*c*) HPV16/18, the first/last smear being positive for HPV16 and/or 18; (*d*) non-16/18 HRHPV, the first/last smear being positive for one or more HRHPV types but not HPV16 or 18; and (*e*) LRHPV, the first/last smear being positive for one or more LRHPV types.

Figure 1. *Continued.*
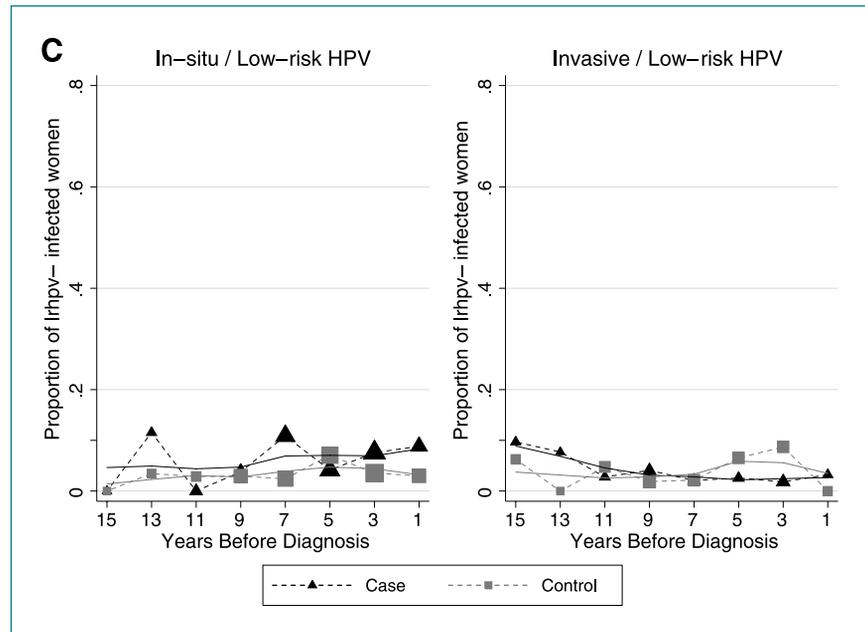
Persistence of HPV16 infection in the first two consecutive smears was defined as: (*a*) negative (both smears negative for HPV16); (*b*) transient (first positive, second negative); (*c*) acquired (first negative, second positive); or (*d*) persistent (both smears positive for HPV16).

Attributable risk proportions and 95% confidence intervals (95% CI) were calculated based on ORs obtained from the conditional logistic regression models (12). All ORs were estimated using STATA version 10 (Stata Corp.).

The study was approved by the Karolinska Institutet Ethics Review Board, which also determined that informed consent from the participants was not required.

Results

Characteristics of the participants

Characteristics of the 515 CIS and 315 SCC cases and their matched control women are described in Table 1. The CIS cases were younger at time of diagnosis (median age, 33; range, 18-64 years) compared with the SCC cases (median age, 40; range, 24-81 years). For CIS cases and controls, the median study time from the first smear to diagnosis of the case was around 5 years (range, 0.03-17.5 years). The time elapsed from the last smear to date of diagnosis was 0.6 years for CIS cases, giving an average interval of 4.7 years between the first and last smear. For CIS controls, this interval was around 3 years. The study time from the first smear for SCC cases and controls was around 7 years (range, 0.01-17.8 years). The time elapsed from the last smear to date of diagnosis was 1.8 years for SCC cases and 2.8 years for their controls. This corresponded to an interval of around 5.5 years between the first and last smear for

cases and just under 5 years for controls. The number of smears registered during follow-up was fairly similar for all women, with a median of 2 (range, 1-15). In the persistence analysis, the median time interval between the first and the second smear was 3 years (range, 0.13-11.6 years), which is the recommended screening interval in Sweden. This interval was very similar in the different exposure categories (negative, transient, acquired, or persistent).

HPV prevalence

The prevalence of any HPV in the first smear at study entry was tripled in CIS and SCC cases, compared with controls (59% and 61% versus 18%, respectively; Table 2). Most of the infections were HPV16/18 in both CIS cases (35%) and SCC cases (44%). Multiple HPV types were present in the first smear of 11% of CIS cases and 9% of SCC cases, but only in 3% to 4% of controls. Table 2 also gives HPV prevalence in the last smear preceding diagnosis of CIS or SCC. The HPV prevalence increased from first to last smear in both CIS and SCC cases, whereas the prevalence among controls diminished slightly.

Among both CIS and SCC cases, the probability of being positive for HPV16/18 at 15 years before diagnosis was around 20% (Fig. 1A). The probability increased as time to diagnosis decreased, to over 40% for CIS cases and 60% for SCC cases one year prior to diagnosis (Fig. 1A). The probability for a non-16/18 HRHPV infection was 10% 15 years prior to and increased to nearly 40% at the time of CIS diagnosis. For SCC cases, this probability remained around 20% from 15 years before to the time of diagnosis. For controls, the probability remained stable over time with around 5% to 10% infected with HPV16/18 or other

Table 3. Conditional logistic regression showing odds ratios and 95% confidence interval of cancer *in situ* and invasive squamous cell carcinoma in the first and last smears among HPV-positive women

| | Reference: all other women* | | Reference: HPV negative women*† | |
| | OR (95% CI) | | OR (95% CI) | |
	First smear	Last smear	First smear	Last smear
Cancer *in situ*				
HPV16/18	6.2 (4.2-9.2)	22.5 (12.3-41.1)	8.5 (5.3-13.7)	37.1 (18.1-76.1)
Non-16/18 HRHPV	3.5 (2.4-5.0)	8.4 (5.4-13.1)	4.4 (2.8-6.8)	15.8 (8.3-30.1)
LRHPV	1.8 (1.0-3.1)	2.0 (1.1-3.7)	2.6 (0.97-6.8)	5.0 (1.4-17.9)
Squamous cell carcinoma				
HPV16/18	12.9 (6.8-24.6)	15.1 (8.0-28.6)	18.6 (9.0-38.9)	28.5 (13.0-62.4)
Non-16/18 HRHPV	2.0 (1.3-3.1)	4.6 (2.7-7.8)	3.0 (1.6-5.5)	16.9 (6.8-42.1)
LRHPV	0.9 (0.4-2.1)	0.7 (0.3-1.6)	1.3 (0.4-4.5)	0.8 (0.1-4.8)

NOTE: Non-16/18 HRHPV denotes any high-risk HPV infection apart from HPV16 or HPV18.

*All estimates were controlled for matching criteria (county, date of entry into cohort, and age at first normal smear).

†ORs further adjusted for all other HPV variables.

high-risk types (Fig. 1A and B). The probability of being infected with LRHPV was similar for CIS/SCC cases and controls, and remained constantly low over time (Fig. 1C).

Risk associations with HPV type

The relative risks increased considerably when using a reference group negative for HPV, as opposed to using a mixed reference group (Table 3). This was particularly evident in the last smear. Being positive for HPV16/18 in the first smear conferred an almost 9-fold (RR, 8.5; 95% CI, 5.3-13.7) increased risk for CIS and 19-fold (RR, 18.6; 95% CI, 9.0-38.9) increased risk for SCC, compared with being HPV negative. These risk associations increased in the last smear, with a more pronounced increase for CIS than for SCC.

The risk of developing CIS or SCC for women infected with non-16/18 HRHPV in the first smear was increased both for CIS (RR, 4.4; 95% CI, 2.8-6.8) and for SCC (RR, 3.0; 95% CI, 1.6-5.5), compared with HPV negative women (Table 3). The risk associations were increased in the last smear, with RRs of 15.8 (95% CI, 8.3-30.1) for developing CIS and 16.9 (95% CI, 6.8-42.1) for developing SCC. Due to a lack of statistical power for HPV types of low prevalence, we could not obtain reliable estimates for the individual non-16/18 HRHPV types.

Separate analyses for HPV16 and 18 were done using a combined end point of CIS and SCC. Women exposed to HPV16 in their first smear had approximately the same risk elevation as those exposed to HPV18 (RR, 11.0; 95% CI, 7.2-16.8; versus RR, 12.2; 95% CI, 4.4-34.1, respectively), compared with HPV-negative women. The risk estimation for HPV16 in the last smear increased substantially (RR, 41.4; 95% CI, 22.7-75.5), whereas the risk for HPV18 increased only modestly (RR, 16.2; 95% CI, 6.1-43.0; data not shown).

HPV type persistence

Among cases, 35% (84 of 241) of CIS and 34% (53 of 155) of SCC were positive for the same HPV type in the first and second smears, compared with only 4% (9 of 241 and 6 of 155, respectively) of the controls. The majority of persistent infections among both cases and controls were attributable to HPV16 (Fig. 2). Compared with women negative for HPV 16 in the first and second smears, being transient conferred no increased risk of CIS and a nonsignificantly increased risk of SCC, whereas having acquired a HPV infection conferred increased risks of both CIS and SCC. Persistence of HPV16 showed a 19-fold increased risk for CIS (RR, 18.5; 95% CI, 6.5-52.9) and a 20-fold increased risk for SCC (RR, 19.5; 95% CI, 4.7-81.7; Table 4).

Attributable risk proportions

In the studied cohort, the HPV16/18 attributable risk proportions (ARP) in the first smear were 29% (95% CI, 24-34%) for CIS and 41% (95% CI, 35-47%) for SCC. The corresponding HPV16/18 ARPs in the last smear were 49% (95% CI, 44-53%) for CIS and 47% (95% CI, 41-53%) for SCC. In the first smear, 20% (95% CI, 15-25%) of CIS cases and 10% (95% CI, 4-16%) of SCC cases were attributable to non-16/18 HRHPV types. These ARPs increased to 34% (95% CI, 29-39%) of CIS cases and 19% (95% CI, 13-25%) of SCC cases in the last smear.

Discussion

This large population-based study prospectively investigated the risk of developing *in situ* and invasive squamous cervical cancer (CIS and SCC) in relation to the presence of all major genital HPV types. It also covered virtually the whole period of active cervical screening in Sweden. Women with a cytologically
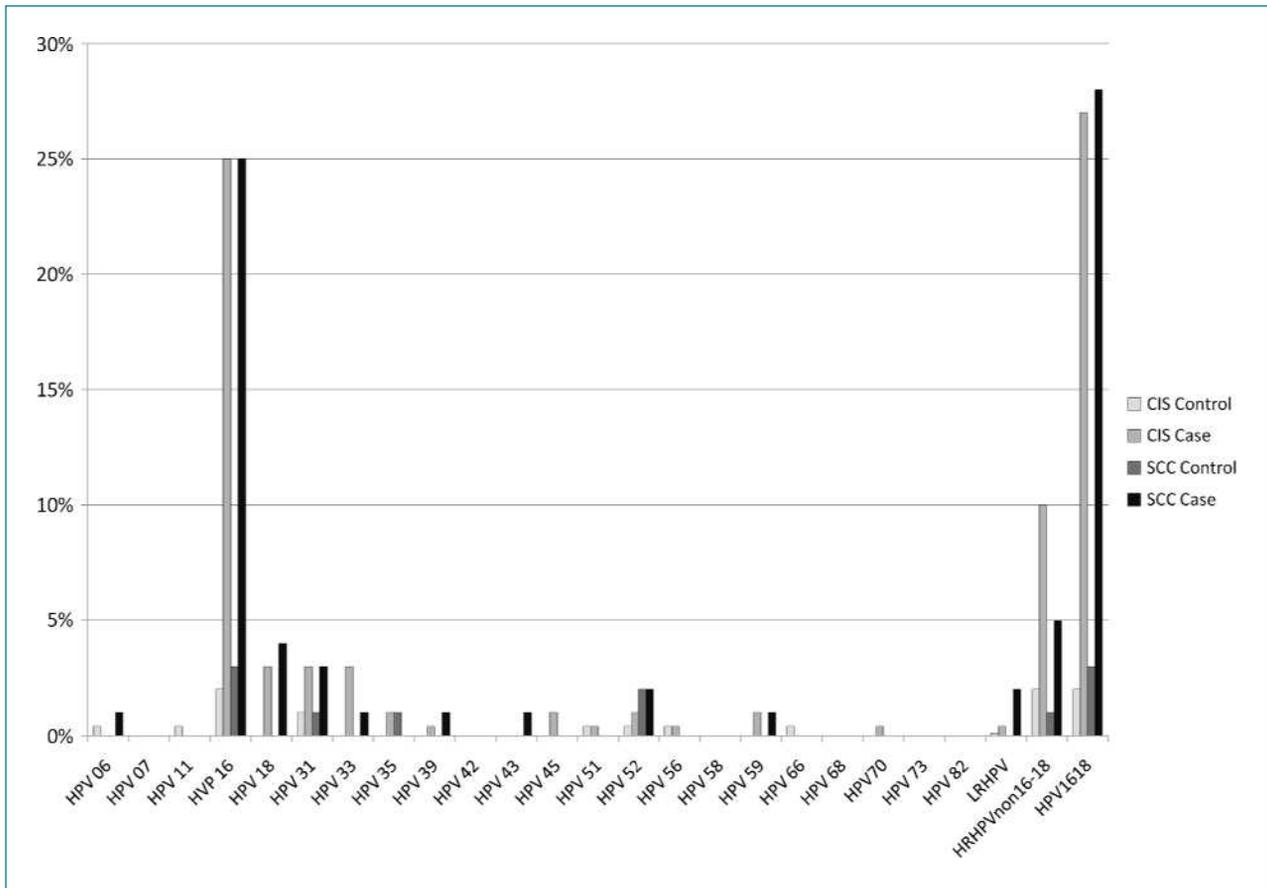
Figure 2. Proportions (%) of women with persistent infections due to different HPV types among 241 cancer *in situ* (CIS) and 155 squamous cell carcinoma (SCC) case women and their matched controls. Twenty-five percent of CIS and SCC cases (60 of 241 and 38 of 155 women, respectively) were persistent for HPV16.

normal smear but infected with HPV16 and/or HPV18 already more than 15 years before diagnosis exhibited a much higher risk for SCC than did HPV-negative women. Our results further suggest that infection with other HRHPV types also carries significant risks, which

may be relevant in the context of HPV vaccination and HPV-based screening.

Much has now been described of the natural history of HPV infection and its causal link to cervical cancer. It is also well known to be a common infection that will

Table 4. Conditional logistic regression showing odds ratios and 95% confidence interval of cancer *in situ* and invasive squamous cell carcinoma among women with transient, acquired, or persistent HPV16 infection compared with those negative for HPV16 in both the first and second smears

Conditional analysis*	CIS (n_{cases} = 241; n_{ctrls} = 241) OR (95% CI)	SCC (n_{cases} = 155; n_{ctrls} = 155) OR (95% CI)
HPV16 status in the first and second smear		
Negative –/–	1.00 (reference)*	1.00 (reference)*
Transience +/–	1.2 (0.5-3.1)	2.6 (0.8-8.2)
Acquisition –/+	7.6 (2.8-20.5)	2.9 (1.2-7.3)
Persistence +/+	18.5 (6.5-52.9)	19.5 (4.7-81.7)

*Estimates were controlled for matching criteria (county, date of entry into cohort, and age at first normal smear).

progress to more severe disease in relatively few women (13). The challenge remains to disentangle which women are at future clinical risk, and identify robust markers of disease progression (14). Although the knowledge base in this area has increased considerably, there have been some limitations to previous studies that have investigated the risks for CIS or SCC imposed by the common oncogenic HPV types. Some studies lack a population-based structure (15, 16) and/or are limited to single municipalities (15, 17-20). Additionally, most studies have limited their HPV typing to one or a few HPV types (15, 17, 20-22) and/or have only assessed HPV types using serologic assays (21, 23). Other prospective population-based studies have included only one outcome (18, 24). Our study significantly advances the prior knowledge because of our large sample size and complete HPV typing that estimated risks associated with the different HPV types for both CIS and SCC within the same population-based cohort comprising all age groups. However, because this study analyzed HPV status only in women who participate in screening, which the large majority of Swedish women do, we cannot draw inference regarding women who do not. Moreover, although they were population based, control women were matched to case women on certain criteria and may therefore not be totally representative of the general female population.

Although a meta-analysis recently validated the use of 6- to 12-month persistence of any HPV infection as a clinical risk marker, the authors noted that few studies in the analysis focused on risks associated with type-specific persistence (25). Furthermore, it is unclear how many cases of invasive cancer were included in the combined end point of the study [CIN2-3/high-grade squamous intraepithelial lesions (HSIL$^+$)]. Other studies have arrived at the same conclusion but have, as yet, only assessed small numbers of CIN1+ or CIN2+ (26-28). Whereas we concur with these findings, we were able to substantiate the concept of persistence also in a large cohort of higher-grade/invasive disease cases. In our study, we observed that being positive for HPV16 in two consecutive smears resulted in close to 17- and 20-fold increased risks for CIS and SCC, respectively, compared with women negative for HPV16 in both smears. The risk was also much higher than that for transiently infected women or for women with a new infection. These estimates show that women with persistent infection with HPV16 are at a substantially increased risk compared with all other women, HPV infected or not. HPV persistence is increasingly being used as an intermediate end point of invasive cervical cancer, both in HPV vaccination trials and in cervical screening strategies. However, this strategy is not yet universally accepted. Our study provides prospective evidence directly supporting the idea that HPV persistence is indeed an important event in the pathway of cervical carcinogenesis.

It should be noted at this point that although our study design allowed us to prospectively study cervical dis-

ease, it could not distinguish between persistent or recurrent infection. Because the time interval between two smears in this study was on average three years, we acknowledge that some of our persistent infections may be re-infections with the same type, although many participants in our study were over the age of 30 and thus past the peak in HPV infection incidence. Due to the time interval between smears, we also emphasize that these findings should not be interpreted in a strict natural history sense, and it was not the aim of this study to describe such a context.

The risk estimates conferred by other (non-16/18) HRHPV types in the first smear were lower for SCC, compared with the risk associated with having a first smear positive for HPV16/18. However, non-16/18 HRHPV types in the last smear imposed considerably higher risk for future CIS, and even SCC development, compared with HPV negativity. Hence, the role of these HPV infections may be important. Indeed, one study found a higher risk of developing CIN2+ among HPV31- and HPV33-positive women than among HPV18-positive women (relative to HPV-negative women; ref. 24). The added risk for cervical cancer conveyed by non-16/18 HRHPV types has direct implications for the efficacy of HPV vaccines. Whereas some evidence exists that a certain degree of cross-protection can occur with the current HPV vaccines (1, 3, 29), this seems to be limited to those HPVs that are phylogenetically related to HPV16 and 18, particularly HPV31, HPV45, and HPV52 (30). It is therefore likely that current vaccines will only partially protect against oncogenic HPV types other than HPV16/18.

We estimate that 41% to 47% of SCC cases in this cohort were attributable to HPV16/18, and thus potentially preventable through current HPV vaccines. The corresponding figure for CIS we estimate to be 30% to 50%, which is in line with a previous study that found a 39% reduction in CIN2+ by removal of HPV16/18 from the Swedish population (24). Furthermore, we found that other remaining high-risk types accounted for at least 10% to 20% of all SCC in our cohort, which illustrates the benefit that could be derived from cross-protection or future expansion with inclusion of additional HPV types in the vaccines. Our ARPs may be overestimates due to issues of population coverage, compliance, and a lower likely efficacy of the vaccine in the general population than in clinical studies. In contrast, our reliance on women's cervical smears rather than on their diagnostic samples, and/or lower sensitivity in analyses based on archival smears, could mean that we underestimated the true values. Extensive typing of tumor tissue has shown near 100% prevalence of HPV DNA in squamous cervical lesions, 70% of which have been attributed to HPV16/18 (13, 31). In the analysis of smears from our case women, around 20% remain HPV negative even in the last smear before diagnosis. Because HPV is recognized as a necessary factor for squamous cervical

cancer development (32), and all known HPV types were tested for, we conclude that our assay has around 80% sensitivity for archival smear HPV typing. This sensitivity did not vary according to how long the smears had been stored in the archive (data not shown). Despite the somewhat limited sensitivity, we still consider our estimates of relative contributions of different HPV types to be of value because they offer a complementary perspective to that derived from tumor tissue, albeit a conservative one.

In summary, our population-based study provides quantification of HPV-type-specific and HPV-persistence-specific cervical cancer risks. We also provide prospective evidence for a strong role of non-16/18 HRHPV types in both CIS and SCC development. The ability for current vaccines to exhibit cross-protective properties would no doubt show considerable benefit to the community, as would the introduction of a further vaccine(s) against other HRHPV types.

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