DOI: 10.1093/jnci/djq356
Advance Access publication on September 14, 2010.

ARTICLE

Long-term Absolute Risk of Cervical Intraepithelial Neoplasia Grade 3 or Worse Following Human Papillomavirus Infection: Role of Persistence

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Manuscript received September 23, 2009; revised January 20, 2010; accepted August 16, 2010.

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Background Infection with high-risk human papillomavirus (HPV) is the main cause of high-grade cervical intraepithelial neoplasia (CIN) and cancer. It has been suggested that information about high-risk HPV type–specific infection might make cervical cancer screening more effective. Persistent HPV infection could also be a useful screening marker. We estimated the long-term risk of high-grade CIN after one-time detection of high-risk HPV DNA and after persistent infection with individual high-risk HPV types.

Methods A cohort of 8656 women from the general population of Denmark was examined twice, 2 years apart (first study examination: May 15, 1991, to January 31, 1993; second study examination: October 1, 1993, to January 31, 1995). The women underwent a gynecological examination and cervical cytology and had swabs taken for HPV DNA analysis by the Hybrid Capture 2 and line probe assays. The women were followed up through the nationwide Danish Pathology Data Bank for cervical neoplasia for up to 13.4 years. The absolute risk of developing cervical lesions before a given time was estimated as a function of time.

Results For women with normal cytological findings who were concurrently HPV16 DNA positive at the second examination, the estimated probability of developing CIN grade 3 (CIN3) or worse within 12 years of follow-up was 26.7% (95% confidence interval [CI] = 21.1% to 31.8%). The corresponding risks among those infected with HPV18 was 19.1% (95% CI = 10.4% to 27.3%), with HPV31 was 14.3% (95% CI = 9.1% to 19.4%), and with HPV33 was 14.9% (95% CI = 7.9% to 21.1%). The absolute risk of CIN3 or worse after infection with high-risk HPV types other than HPV16, HPV18, HPV31, or HPV33 was 6.0% (95% CI = 3.8% to 8.3%). The estimated absolute risk for CIN3 or cancer within 12 years of the second examination among women who were HPV16 DNA positive at both examinations was 47.4% (95% CI = 34.9% to 57.5%); by contrast, the risk of CIN3 or worse following a negative Hybrid Capture 2 test was 3.0% (95% CI = 2.5% to 3.5%).

Conclusion HPV16, HPV18, HPV31, and HPV33 infection and especially HPV16 persistence were associated with high absolute risks for progression to high-grade cervical lesions. The results indicate the potential value of genotyping in cervical cancer screening. Given that HPV DNA–negative women retained their low risk of CIN3 or worse for many years, frequent screening of these women may be unnecessary.

J Natl Cancer Inst 2010;102:1478–1488

High-risk human papillomavirus (HPV) types have been shown to be involved in the development of cervical cancer (1). There has thus been increasing interest in the potential clinical use of HPV testing to triage women who have minor cervical cytological changes, to follow up women who are treated with the loop electrosurgical excision procedure for severe cervical neoplasia, and in primary screening against cervical cancer. Although several HPV types have been characterized as high-risk or carcinogenic HPV types, they do not appear to have the same carcinogenic potential (2). Most of the available information about HPV type–specific risks for high-grade squamous intraepithelial neoplasia or cervical cancer comes from prevalence and case–control studies, both of which have a cross-sectional design. The subsequent risk for cervical neoplasia associated with groups or a limited number of HPV types has been addressed in some prospective studies (3,4), but only a few studies have looked at the prospective risk of cervical neoplasia associated with a broader spectrum of individual high-risk HPV types (2,5,6). It has been shown previously that a single positive test for high-risk HPV in a woman with normal cytology is predictive of her subsequent risk for developing high-grade squamous intraepithelial neoplasia or histologically confirmed high-grade cervical intraepithelial neoplasia (ie, cervical intraepithelial neoplasia grade 3 [CIN3]) (7–10). It has been suggested that this benefit of HPV testing could be enhanced by testing for specific

high-risk HPV types (3). In a recent study of women in Costa Rica, Castle et al. (11) reported that short-term persistent infection with a high-risk HPV type (HPV16 in particular) was a strong predictor of cervical intraepithelial neoplasia grade 2 (CIN2) or worse over the subsequent 3–5 years.

We conducted a large, population-based, prospective cohort study to examine the absolute risk for high-grade cervical lesions after one positive test for a high-risk HPV or a persistent infection (defined as two positive tests) with various specific high-risk HPV types in women with normal cytological findings during a follow-up period of more than 13 years.

Subjects and Methods

Study Population

The study cohort consisted of women who were 20–29 years of age at enrollment. The women were selected at random from the general female population of Copenhagen, Denmark. In Denmark, every citizen has a unique 10-digit personal identification number, which is universally used in Danish society and all health registries. These identification numbers, which contain information on sex and date of birth, are registered in the computerized Danish Central Population Register. This register, which is up dated on a daily basis, includes information on vital status, emigration, and current address. The women were invited specifically for this study by mail, and those who did not respond, received a reminder after 4–10 weeks. Between May 15, 1991, and January 31, 1993, we enrolled 11 088 women into the study for the first gynecological examination.

Approximately 2 years after enrollment (from October 1, 1993, to January 31, 1995), the study participants (now 22–32 years of age) were re-invited, in the same order in which they were originally enrolled, for a second gynecological examination. To obtain information on the current address and vital status on all the women in the study, the cohort members were initially linked to the Danish Central Population Register by their unique personal identification numbers. Subsequently, the women were invited by mail to the second study examination. A total of 8656 women (78%) participated in this second examination. At both gynecological examinations, a Pap smear was taken, and a portion of the smear containing ecto- and endocervical cells was placed in a tube containing phosphate-buffered saline and stored at −80°C for subsequent HPV DNA testing. In addition, at both examinations, each woman participated in an interview that was conducted in person by a female nurse. A small proportion of the women were interviewed via telephone. Before entering the study, all participants were informed verbally and in writing about the study, and all participants signed a written informed consent. The study was approved by the national Scientific Ethical Committee and the national Data Protection Board.

The population for the study reported here comprises the 8656 women who participated in both gynecological examinations, and the baseline used in the analyses was the date of the second examination, because we wanted to look at outcomes subsequent to HPV DNA testing results from both study examinations. We excluded 381 women who participated in the second examination only through a telephone interview (ie, they did not undergo a gynecological examination), 193 women with an abnormal smear at baseline, 47 women who had had an abnormal smear within 1 year before baseline, and 356 women for whom no cervical swab was available at the baseline visit (eg, because they were menstruating at the time of the examination or because their cervical swab was inadequate for HPV DNA testing), leaving 7679 women with normal cytological findings at baseline. Finally, we excluded 197 women who did not have any gynecological examination after baseline, leaving 7482 women who were included in the analysis.

Follow-up

After the second examination (study baseline), the cohort was followed up passively through the Pathology Data Bank. Correct linkages between registries were ensured through the personal identification numbers. The existence of the nationwide Central Population Register makes it possible to conduct follow-up studies with virtually no loss to follow-up.

CONTEXT AND CAVEATS

Prior knowledge

Infection with a high-risk type of human papillomavirus (HPV) is the main cause of high-grade cervical intraepithelial neoplasia (CIN) and cancer. However, few studies have looked at the long-term prospective risk of cervical neoplasia associated with a broad spectrum of individual high-risk HPV types or with a persistent HPV infection.

Study design

Population-based prospective cohort study examining the absolute risk for high-grade cervical lesions after one positive test for a high-risk HPV type or two positive tests for the same high-risk HPV types (a persistent infection) in women with normal cytological findings with follow-up of more than 13 years.

Contribution

Infection with HPV16 was the most prevalent and had the greatest tendency to persist and the highest probability for progression when it persisted, followed by infection with HPV18, HPV31, and HPV33. The main predictor of subsequent risk of CIN3 or worse was HPV16 persistence. One positive test and persistence for high-risk HPV types other than HPV16, HPV18, HPV31, and HPV33 were associated with low absolute risks of CIN3 or worse that lasted for years. HPV negative women stayed at very low risk of CIN3 or worse.

Implications

These findings may be useful in the development of more specific cervical cancer screening methods, identify issues that need to be resolved to obtain the greatest clinical value from HPV testing, and/or be of value in the development of new generations of prophylactic HPV vaccines and suggest that cervical cancer screening intervals for HPV-negative women could be prolonged.

Limitations

The rates of progression of some HPV types after persistence may have been overestimated. The duration of persistent infection and its role in the risk of progression was not assessed. Some of the infections defined as persistent might have been re-infections with the same HPV type, which would have resulted in an underestimation of the risk of CIN after HPV persistence.

From the Editors

The Pathology Data Bank is a nationwide pathology register that contains information on all cervical cytology (organized and opportunistic, normal and abnormal) and all cervical biopsy specimens, cones, and hysterectomies (normal and abnormal histology) performed in Denmark for the last 20 years. Clinical communication between pathology departments and the Pathology Data Bank is ensured through an online real-time data reporting system. Abnormal cervical diagnoses are usually reported as atypia, mild dysplasia, moderate dysplasia, severe dysplasia, or carcinoma in situ. The histological diagnoses were translated into CIN nomenclature as follows: moderate dysplasia was categorized as CIN2 and severe dysplasia and carcinoma in situ were categorized as CIN3. In Denmark, cervical cancer screening by means of cervical cytology is recommended every 3 years beginning at the age of 23 years (HPV testing has not yet been introduced for primary cervical cancer screening in Denmark).

Using the personal identification number as the key identifier, we linked the cohort to the Pathology Data Bank and followed it until March 6, 2007 to obtain information on all cervical cytology and histology and to identify all cervical pathological lesions and diagnostic or treatment procedures. Because the HPV DNA testing took place several years after the study examinations were conducted, the women were unaware of the results obtained in the study, and results of the study HPV DNA testing were not used for referrals for colposcopy, treatment, or clinical management of the women.

HPV DNA Detection

The HPV DNA testing strategy has been described previously (9). Due to the fact that the specimens were collected into a medium (phosphate-buffered saline) that is not recommended for the Hybrid Capture 2 (HC2) test, the specimens were subjected to a conversion protocol to allow HC2 testing, as described previously (9). Briefly, a 75-μL aliquot of each sample was denatured in NaOH and then subjected to HPV DNA testing with the high-risk probe of the HC2 assay (Qiagen, Hilden, Germany), according to the manufacturer's instructions, using a robot platform device (Rapid Capture System 1; Qiagen, Hilden, Germany) that can simultaneously process four 96-well microtiter plates at the same time. Each HC2 microtiter plate included three negative (negative calibrator) controls and three positive (high-risk calibrator) controls. These calibrators are included in the test kit with a defined amount of HPV DNA (positive calibrator) and HPV DNA-free buffer (negative calibrator) to determine for each individual test the cut-off relative light value. It also included two wells containing human cervical cancer C33A cells (HPV DNA negative, at 10^4 cells per well) and two wells containing human cervical cancer SiHa cells (HPV16 DNA positive, at 10^4 and 10^5 cells per well) to monitor the performance of the HC2 assay. The cell lines were obtained from the American Type Culture Collection (Manassas, VA) and were expanded within 3 months to obtain enough frozen samples that could be used throughout the study. No further verification analysis was conducted. The HC2 assay is based on hybridization, in solution, of long synthetic RNA probes complementary to the genomic sequence of 13 high-risk (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) (12,13). Denatured HPV DNA present in the biological specimen was hybridized in solution with only the high-risk probe mix allowing the formation of specific HPV DNA–RNA hybrids. These hybrids were captured by antibodies bound to the wells of a microtiter plate that recognize RNA–DNA hybrids. The immobilized hybrids were then reacted with an alkaline phosphatase–labeled anti-DNA–RNA monoclonal antibody, after which the plate was washed and the wells were incubated with CDP-Star (Tropix PE, Bedford, MA), a chemiluminescent alkaline phosphatase substrate. Dephosphorylation of this substrate produces light, which was measured with the use of luminometer (Qiagen). The intensity of the emitted light, expressed as relative light units, is proportional to the amount of target DNA present in the specimen and provides a semiquantitative measure of the viral load. Samples were considered positive for high-risk HPV if they attained or exceeded the threshold of 1.0 pg/mL of HPV DNA recommended by the United States Food and Drug Administration (13), which corresponds to 1.0 relative light units. All laboratory personnel were fully blinded to the cytological diagnoses and the results of the follow-up examinations. Retesting of 10% of the samples (randomly chosen) by repeating the HC2 test with the high-risk probe gave virtually identical results.

HPV genotypes for samples that were positive for high-risk HPV DNA in the HC2 assay were determined with the use of a polymerase chain reaction–based line probe assay (LiPA) as previously described (14). Briefly, total DNA was isolated from the remaining denatured cervical samples with the use of a MagNAPure device (Roche, Indianapolis, IN) and analyzed with the use of the INNO-LiPA v2HPV prototype assay (provided by Innogenetics, Inc, Gent, Belgium) according to the manufacturer's instructions. All polymerase chain reaction manipulations were performed in a laboratory that was separate from the other laboratory rooms according to good laboratory practice guidelines. An aliquot of the amplified polymerase chain reaction product was hybridized to an LiPA hybridization strip, which allows the simultaneous detection of 24 HPV genotypes on one strip, with the use of an Auto-LiPA device (both from Innogenetics, Inc). The strips were analyzed on a flatbed scanner with the use of LiRAS prototype software (Innogenetics, Inc), which displays the patterns and relative intensity of positive bands as arbitrary gray-tone values between 0.1 and 1.0. The INNO-LiPA v2HPV test can be used to identify 24 HPV genotypes (HPV6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, and 74); the hybridization strips also contained a consensus probe that could hybridize to an extended range of HPV types other than the 24 types on the strip. Samples that were found to be positive in the LiPA assay but for which a specific HPV type could not be identified were excluded from this analysis.

Statistical Analysis

The women were followed up from baseline until March 6, 2007 for incident abnormal cervical cytological or histological findings. When looking at a particular outcome (atypia or worse, CIN2 or worse, or CIN3 or worse) in relation to each of the high-risk HPV types separately, we calculated simple proportions of women who developed the specific cervical lesion during the entire follow-up period without taking into account person-time at risk. This analysis also grouped the HPV types in species groups. Based on analysis of the HPV genome, an HPV phylogentic tree has previously been

developed (1). The genital HPV types belong to the genus alpha papillomaviruses and lower-order clusters are described as species (1).

The absolute risk of developing cervical lesions was also estimated as a function of time by assuming a model of piecewise constant intensity and taking into account the fact that the exact time points when the cervical lesions developed were not known; the only information that was available was either that an event had occurred between two dates of testing or that no event had occurred by the last test date (interval-censured observations) (15). The occurrence of cervical abnormalities was assumed to be constant within 0–3, 3–4, 4–5, 5–6, 6–7, 7–8, and 8 or more years of follow-up time. In the analyses related to persistence of individual HPV types, we used three time periods (0–3, 3–6, and 6 or more years) because of small numbers. Pointwise 95% confidence intervals (CIs) were calculated using the estimated variance of the integrated intensity at the end of each follow-up interval. In this study, persistence of a specific HPV type was defined as being positive for that HPV type at both study examinations. Likewise, high-risk HPV persistence was defined as being HC2 test positive at both examinations. Incident infections (newly detected) with a specific HPV type was defined as being negative for the specific HPV type at the first study examination and positive for that HPV type at the second examination.

Results

Of the 7482 women with a normal Pap smear at baseline who were included in the study, 1281 (17.1%) were positive for high-risk HPV DNA by the HC2 assay. Among the women who were high-risk HPV DNA positive by HC2, the specific HPV type could not be identified in 59; the remaining 1222 women with an identified HPV type(s) were included in this analysis. Among the women in the study population, the median age at baseline was 28 years (range = 22–32 years); the median age of HPV DNA–positive women was 27.8 years (range = 22–32 years) and of HPV DNA–negative women, 26.9 years (range = 22–32 years). Median follow-up for study participants was 12.9 years (range = 12.1–13.4 years). HPV DNA–positive women with no cervical abnormalities during the follow-up period had a median of four Pap smears (range = 1–15 Pap smears) after baseline, as did HPV DNA–negative women (range = 1–14 Pap smears).

We examined the distribution of HPV types at baseline (Table 1). The most common HPV type was HPV16, which accounted for 24.2% of HPV DNA–positive women, followed by HPV31 (20.5%), HPV52 (20.1%), HPV51 (14.4%), HPV33 (13.4%), HPV39 (11.3%), HPV45 (11.0%), and HPV56 (10.0%). The frequencies of the remaining high-risk HPV types were less than 10%. Some HPV types often occurred as single infections (ie, HPV16 [41.6%], HPV59 [38.1%], HPV45 [34.8%], and HPV51 [33.0%]), whereas other types occurred only rarely as single infections (ie, HPV66 [5.0%], HPV53 [11.0%], and HPV58 [13.5%]).

Proportions of Women Who Developed Atypia, CIN2, or CIN3 or Worse During Follow-up After Having One Positive Test for Specific High-Risk HPV Types

Table 2 presents the proportions of women who developed atypia or worse on cytology and CIN2 or worse on histology during the entire follow-up period in the total study population of HPV DNA–positive women and in women with a single HPV type, women with a given HPV type plus one or more of the other high-risk HPV types except HPV16, and women with a given HPV type plus HPV16. In the total study population, women who were positive for any of the high-risk HPV types at baseline had a relatively high likelihood of having a subsequent abnormal Pap smear

Table 1. Frequency of specific high-risk (HR) human papillomavirus (HPV) types at baseline, overall, as a single infection, and with other HR HPV types without and with HPV16*

HPV species and type	All women with a given HR HPV type (n = 1222), N (%)	Women with a given HPV type alone, N (%)	Women with a given HPV type plus other HR HPV type(s) (except HPV16), N (%)	Women with a given HPV type plus HPV16 with or without other HR HPV type(s), N (%)
Alpha 9				
HPV16	296 (24.2)	123 (41.6)	173 (58.4)	NA
HPV31	250 (20.5)	51 (20.4)	152 (60.8)	47 (18.8)
HPV33	164 (13.4)	47 (28.7)	86 (52.4)	31 (18.9)
HPV35	48 (3.9)	11 (22.9)	32 (66.7)	5 (10.4)
HPV52	246 (20.1)	64 (26.0)	136 (55.3)	46 (18.7)
HPV58	89 (7.3)	12 (13.5)	68 (76.4)	9 (10.1)
Alpha 7				
HPV18	116 (9.5)	26 (22.4)	69 (59.5)	21 (18.1)
HPV39	138 (11.3)	28 (20.3)	89 (64.5)	21 (15.2)
HPV45	135 (11.0)	47 (34.8)	75 (55.6)	13 (9.6)
HPV59	63 (5.2)	24 (38.1)	31 (49.2)	8 (12.7)
HPV68	117 (9.6)	26 (22.2)	77 (65.8)	14 (12.0)
Alpha 6				
HPV53	109 (8.9)	12 (11.0)	76 (69.7)	21 (19.3)
HPV56	122 (10.0)	36 (29.5)	65 (53.3)	21 (17.2)
HPV66	121 (9.9)	6 (5.0)	87 (71.9)	28 (23.1)
Alpha 5				
HPV51	176 (14.4)	58 (33.0)	84 (47.7)	34 (19.3)

* NA = not applicable.

Table 2. Number and proportion of women who developed atypia or worse (by cytology) or cervical intraepithelial neoplasia grade 2 (CIN2) or worse (by histology) during the entire follow-up period of 13.4 years in relation to human papillomavirus (HPV) type detected at baseline among cytologically normal Danish women

	Proportion developing atypia or worse during follow-up				Proportion developing CIN2 or worse during follow-up			
HPV species and type	**All women with the given HPV type**	**Women with the given HPV type alone**	**Women with the given HPV type plus other high-risk HPV types except HPV16**	**Women with the given HPV type plus HPV16 with or without other high-risk HPV types**	**All women with the given HPV type**	**Women with the given HPV type alone**	**Women with the given HPV type plus other high-risk HPV types except HPV16**	**Women with the given HPV type plus HPV16 with or without other high-risk HPV types**
	N (%)	%	%	%	N (%)	%	%	%
Alpha 9								
HPV16	113 (38.2)	40.7	36.4	NA	82 (27.7)	28.5	27.2	NA
HPV31	77 (30.8)	29.4	27.6	42.6	49 (19.6)	15.7	17.1	31.9
HPV33	7 (34.8)	31.9	31.4	48.4	36 (22.0)	19.1	16.3	41.9
HPV35	17 (35.4)	27.3	40.6	20.0	11 (22.9)	18.2	28.1	0
HPV52	73 (29.7)	20.3	30.9	39.1	39 (15.9)	4.7	19.9	19.6
HPV58	24 (27.0)	16.7	29.4	22.2	18 (20.2)	16.7	22.1	11.1
Alpha 7								
HPV18	38 (32.8)	23.1	37.7	28.6	24 (20.7)	15.4	23.2	19.0
HPV39	30 (21.7)	14.3	22.5	28.6	16 (11.6)	3.6	10.1	28.6
HPV45	26 (19.3)	14.9	21.3	23.1	15 (11.1)	8.5	10.7	23.1
HPV59	11 (17.5)	4.2	19.4	50.0	6 (9.5)	0	6.5	50.0
HPV68	16 (13.7)	3.8	15.6	21.4	8 (6.8)	0	7.8	14.3
Alpha 6								
HPV53	21 (19.3)	0	17.1	38.1	12 (11.0)	0	7.9	28.6
HPV56	22 (18.0)	19.4	13.8	28.6	9 (7.4)	2.8	9.2	9.5
HPV66	27 (22.3)	16.7	17.2	21.8	18 (14.9)	0	12.6	25.0
Alpha 5								
HPV51	27 (19.3)	17.2	16.7	29.4	18 (10.2)	8.6	6.0	23.5

*NA = not applicable.

(atypia or worse), ranging from 38.2% for HPV16–positive women to 13.7% for HPV68–positive women. Women who were HPV16 positive at baseline had the highest likelihood of developing CIN2 or worse. This finding was independent of whether HPV16 occurred as a single infection (28.5%) or together with other high-risk HPV types (27.2%). Of the other high-risk HPV types found as single infections, the risk for CIN2 or worse was highest for the HPV types in the alpha 9 group (except for HPV52) and for HPV18 in the alpha 7 group. No CIN2 or worse lesions were found following a single infection with HPV53, HPV59, HPV66, or HPV68. Furthermore, for most, but not all (ie, HPV18, HPV35, HPV52, HPV56, and HPV58) HPV types, women with multiple infections had a higher likelihood of developing CIN2 or worse when they were infected with the specific HPV type together with HPV16 than together with other high-risk HPV types (Table 2).

The proportions of women with normal cytology who developed CIN3 or worse on histology according to the HPV type detected at baseline are presented in Table 3. Women who were positive for HPV16 at baseline had a similar likelihood of developing CIN3 or worse regardless of whether they were infected with HPV16 alone (26%) or in conjunction with other high-risk HPV types (24.9%). This pattern was also observed, albeit to a lesser extent, for HPV18: 15.4% of women with a single HPV18 infection at baseline, 18.8% of women infected with HPV18 and other high-risk HPV types (except HPV16) at baseline, and 19% of women infected with HPV18 and HPV16 at baseline developed CIN3 or worse within a maximum follow-up time of 13.4 years. The absolute risks for single infections with HPV types other than HPV16 and HPV18 were substantially lower (eg, HPV33 [12.8%], HPV31 [9.8%], HPV35 [9.1%], HPV58 [8.3%], HPV45 [6.4%]). For the majority of high-risk HPV types other than HPV16 and HPV18, women who were co-infected with a specific high-risk HPV type plus HPV16 were more likely to develop CIN3 or worse than women who were infected with the specific high-risk HPV type alone or in conjunction with other high-risk HPV types (excluding HPV16). There were no diagnoses of CIN3 or worse in women who were positive for HPV39, HPV53, HPV59, HPV66, or HPV68 alone at baseline, and only rare occurrences of CIN3 or worse among women who were positive for HPV52 or HPV56 alone (<5% of women during the entire follow-up period).

Absolute Risk of CIN3 or Worse According to Years of Follow-up After One Positive Test for a Specific High-Risk HPV Type

We estimated the absolute risk of developing CIN3 or worse after testing positive for various high-risk HPV types in relation to follow-up time (Figure 1). For women who had normal cytology and concurrently tested positive for HPV16 DNA at baseline (with or without testing positive for other high-risk HPV types), the estimated probability of developing CIN3 or worse within the first 12 years of follow-up was 26.7% (95% CI = 21.1% to 31.8%). The estimates according to follow-up time were virtually identical when HPV16 occurred alone, without co-infection with other high-risk HPV types (data not shown). The absolute risks of CIN3 or worse after infection with HPV18, HPV31, and HPV33 (with

Table 3. Number and proportion of women who developed cervical intraepithelial neoplasia grade 3 (CIN3) or worse during the entire follow-up period of 13.4 years in relation to human papillomavirus (HPV) type detected at baseline among cytologically normal Danish women*

	Proportion developing CIN3 or worse during follow-up			
HPV species and type	All women with the given HPV type	Women with the given HPV type alone	Women with the given HPV type plus other high-risk HPV types except HPV16	Women with the given HPV type plus HPV16 with or without other high-risk HPV types
	N (%)	%	%	%
Alpha 9				
HPV16	75 (25.3)	26.0	24.9	NA
HPV31	40 (16.0)	9.8	13.8	29.8
HPV33	30 (18.3)	12.8	12.8	41.9
HPV35	7 (14.3)	9.1	18.8	0
HPV52	31 (12.6)	4.7	14.7	17.4
HPV58	14 (15.7)	8.3	17.6	11.1
Alpha 7				
HPV18	21 (18.1)	15.4	18.8	19.0
HPV39	14 (10.1)	0	9.0	28.6
HPV45	12 (8.9)	6.4	8.0	23.1
HPV59	6 (9.5)	0	6.5	50.0
HPV68	5 (4.3)	0	5.2	7.1
Alpha 6				
HPV53	6 (5.5)	0	3.9	14.3
HPV56	7 (5.7)	2.3	7.7	4.8
HPV66	14 (11.6)	0	8.0	25.0
Alpha 5				
HPV51	16 (9.2)	6.9	4.8	23.5

* NA = not applicable.

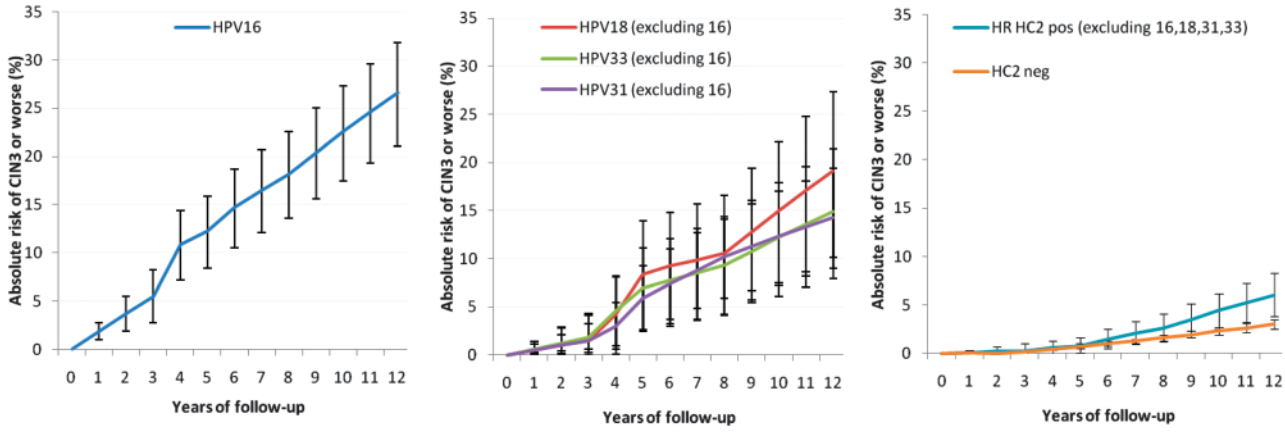

Figure 1. Absolute risks of cervical intraepithelial neoplasia grade 3 (CIN3) or worse after infection with different high-risk human papillomavirus (HPV) types in women with normal cytological findings at baseline. **Error bars** correspond to 95% confidence intervals. HR HC2 positive = positive to high-risk HPV types as measured by the Hybrid Capture 2 test. HC2 neg = HC2 negative.

no HPV16 co-infection and with or without infection with other high-risk HPV types) were similar during the first 8 years of follow-up and lower than for HPV16. In the last part of the follow-up period (ie, from year 8 onward), the risk of CIN3 or worse tended to be higher among those infected with HPV18 than among those infected with HPV31 or HPV33. We observed a delay in the time to development of CIN3 or worse after infection with HPV18 (or HPV31 or HPV33) compared with after infection with HPV16. After 12 years of follow-up, the absolute risk of CIN3 or worse among those infected with HPV18 was 19.1% (95% CI = 10.4% to 27.3%), with HPV31 was 14.3% (95% CI = 9.1% to 19.4%), and with HPV33 was 14.9% (95% CI = 7.9% to 21.1%). The absolute risks of CIN3 or worse after infection with high-risk HPV types other than HPV16, HPV18, HPV31, and HPV33 was 6.0% (95% CI = 3.8% to 8.3%). At 12 years of follow-up, the lowest risk of CIN3 or worse was for women who were negative for high-risk HPV types at baseline (absolute risk = 3.0%, 95% CI = 2.5% to 3.5%).

Absolute Risk of CIN3 or Worse Associated With Persistence of Specific High-Risk HPV Types

We also examined the risk of CIN3 or worse in women who were persistently positive for HPV16 or other high-risk HPV types on two occasions (eg, women who were HPV16 DNA positive at baseline and at 2 years before baseline in this study). Figure 2 illustrates for specific high-risk HPV types, the type-specific prevalence (among HPV-positive women) the percentage of women with persistent infection, and the percentage of women who developed CIN3 or worse among those with a persistent infection. In general, HPV types in the alpha 9 group were more prevalent at baseline, persisted more often, and were more likely to result in progression to CIN3 or worse (given persistence) compared with HPV types in the alpha 5, 6, and 7 groups. For example, 29.4% of HPV16 infections were persistent, and 46% of women with a persistent HPV16 infection developed CIN3 or worse during follow-up. HPV35, with a low prevalence (3.9%), had a persistence rate that was similar to that of HPV31 (18.8% vs 21.6%) but had the lowest tendency of all alpha 9 group members to progress to CIN3 or worse. In the alpha 7 group, HPV18 had a higher persistence rate and a higher progression rate, given persistence, compared with the other HPV types. This was also the case for HPV51 in the combined alpha 5 and 6 groups. No women with persistent infections with HPV59 or HPV68 in the alpha 7 group or with HPV56 or HPV53 in the combined alpha 5 and 6 groups developed CIN3 or worse during follow-up. We also examined the proportion of women who developed CIN3 or worse after persistence of a given HPV type in women who were HPV16 negative at baseline. We found that the absolute risks of CIN3 or worse associated

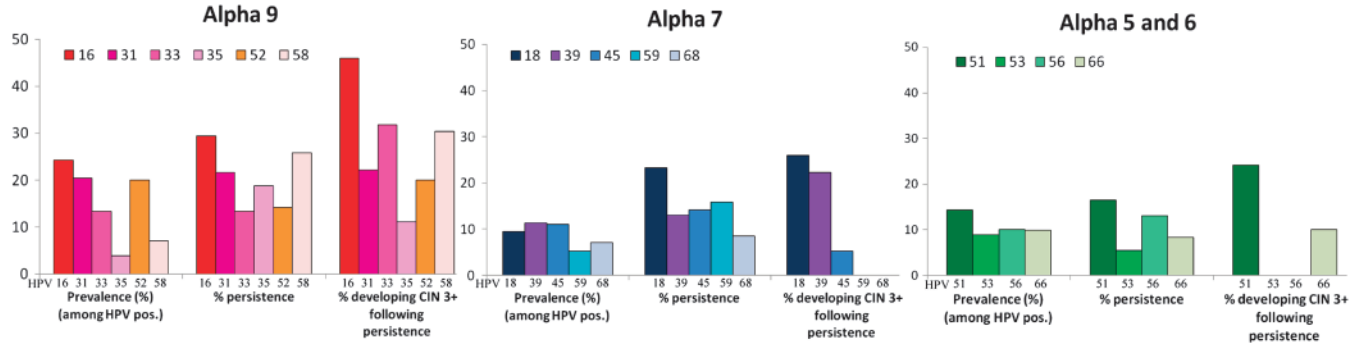

Figure 2. High-risk human papillomavirus (HPV) type–specific prevalence of infection, percentage of women with a persistent infection, and percentage of women with persistent infection who developed cervical intraepithelial neoplasia grade 3 or worse during follow-up. CIN3+ = cervical intraepithelial neoplasia grade 3 or worse.

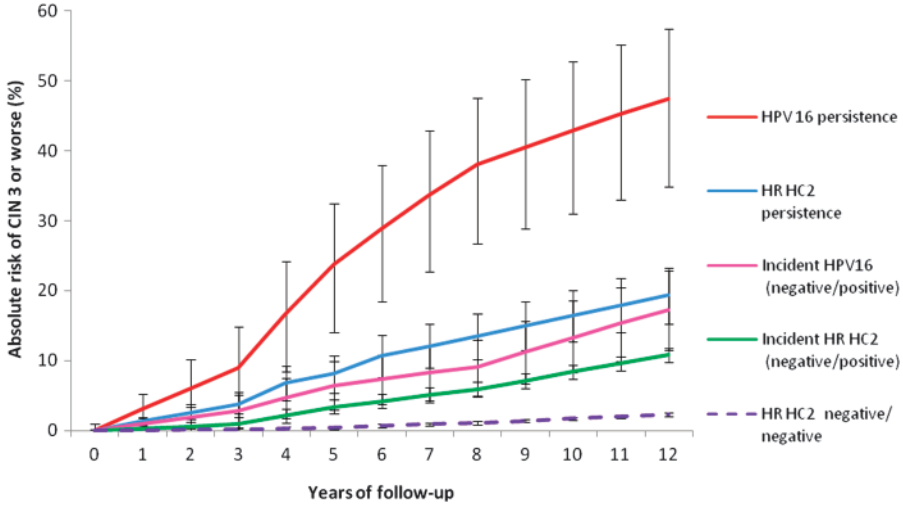

Figure 3. Absolute risks of developing cervical intraepithelial neoplasia grade 3 (CIN3) or worse in women with normal cytological findings at baseline in relation to various measures of human papillomavirus (HPV) status. HPV16 persistence = positive to HPV16 at the first and at the second study examination. HR HC2 persistence = positive for high-risk HPV types as measured by the Hybrid Capture 2 at the first and the second study examination; incident HPV16 = negative to HPV16 at the first study examination and positive to HPV16 at the second study examination; incident HR HC2 = negative for high-risk HPV by the HC2 assay at the first and positive at the second study examination; HR HC2 negative/negative = negative for high-risk HPV by the HC2 assay at the first and the second study examination, **Error bars** correspond to 95% confidence intervals.

with persistent infections were substantially lower than the absolute risk of CIN3 or worse in the overall analysis (data not shown).

Finally, we estimated the probability of developing CIN3 or worse according to HPV DNA test status at the first and the second study examinations in relation to follow-up time (Figure 3). The absolute risks of CIN3 or worse at 3, 5, and 12 years of follow-up among women with two positive tests for HPV16 (ie, HPV16 persistence) were 8.9% (95% CI = 2.5% to 14.9%), 23.8% (95% CI = 14.1% to 32.4%), and 47.4% (95% CI = 34.9% to 57.5%), respectively. The corresponding absolute risks for CIN2 or worse at 3, 5, and 12 years after two positive tests for HPV16 were 11.3 (95% CI = 4.2% to 17.8%), 24.7% (95% CI = 14.9% to 33.4%), and 50.9% (95% CI = 38.3% to 60.9%), respectively (data not shown). For comparison, we estimated the absolute risks for CIN3 or worse during follow-up among women with an incident or newly detected HPV16 infection (ie, HPV16 negative at the first examination and HPV16 positive at the second examination), women with an incident high-risk HPV infection (ie, negative for high-risk HPV by the HC2 assay at the first examination and positive at the second examination), women with a persistent high-risk HPV infection (ie, positive for high-risk HPV by the HC2 assay at both examinations), and women who were negative for high-risk HPV by the HC2 assay at both examinations (Figure 3). After 12 years, women with an incident HPV16 infection had virtually the same absolute risk of CIN3 or worse (17.3%; 95% CI = 11.5% to 22.8%) as women who had a persistent high-risk HPV infection (19.3%; 95% CI = 15.2% to 23.3%). Only two (3%) of 73 women who had a persistent infection with a specific high-risk HPV type other than HPV16, HPV18, HPV31, or HPV33 developed CIN3 or worse during follow-up (data not shown). This frequency was similar to that among women who were HPV negative at the first and second study examinations (2.3%; 95% CI = 1.9% to 2.8%).

Discussion

This population-based prospective cohort study of 7482 women with normal cytology from the general population, with follow-up for up to 13.4 years through a routine screening system with virtually no loss to follow-up, provides estimates of the absolute risks for high-grade cervical lesions (ie, CIN2, CIN3, or worse) after infection with specific high-risk HPV types and estimates of the long-term absolute risk for high-grade CIN or cancer) after persistent infection with various high-risk HPV types. We found that HPV16 was the HPV type with the greatest carcinogenic potential. More than 25% of women with normal cytological findings who were HPV16 DNA positive at study baseline developed CIN3 or worse within 12 years. Although women with normal cytology who were positive for other high-risk HPV types, including HPV18, HPV31, HPV33, and HPV58, also had high risks for developing high-grade cervical lesions, those risks were much lower than those associated with HPV16 DNA positivity.

It can be difficult to determine the risk of developing high-grade cervical lesions that is associated with a specific HPV type in women with multiple HPV infections because the risk might be influenced by co-infection with other HPV types. Therefore, we also examined the absolute risk of high-grade cervical lesions after a single infection with a specific HPV type in an attempt to isolate the effect of the individual HPV type. Women who had a single infection with HPV16 (26%) or HPV18 (15.4%) had the greatest absolute risk for subsequent CIN3 or worse, followed by women who had a single infection with HPV33 (12.8%), HPV31 (9.8%), HPV35 (9.1%), HPV58 (8.3%), or HPV45 (6.4%). It is noteworthy that CIN3 or worse was not observed during the follow-up time up to 13.4 years after a single infection with five other high-risk HPV types (ie, HPV39, HPV59, HPV68, HPV53, and HPV66). Our results for HPV16 and HPV18 are similar to those of cohort studies in the United States with 10 years of follow-up (3) and in Costa Rica with 5–7 years of follow-up (2), in which HPV16 was consistently the HPV type that was associated with the highest risk for CIN3 or worse, followed by HPV18. In a recent cohort study from Sweden (6), the absolute risk of CIN3 or worse that was associated with HPV18 was much lower than the absolute risk associated with HPV16, and women with an HPV33 infection had the same high absolute risk of CIN3 or worse as women with an HPV16 after 4 years of follow-up. In our previous report on the absolute risk of CIN3 or worse in women with normal cytology who concurrently tested positive for high-risk HPV in the HC2 assay, we found that a single positive test is a good predictor of

high-grade CIN (9). In this study, the detection of HPV16 DNA, and of HPV18 DNA, allowed a better stratification of women with regard to their subsequent risk of CIN3 or worse. In line with this finding, it has been suggested that women who test positive for HPV type 16 or 18 could benefit from immediate colposcopy (16).

Because viral persistence rather than transient viral infection plays a crucial role in cervical carcinogenesis (17,18), it could be hypothesized that measures of viral persistence would increase the accuracy of cervical cancer screening systems. Few studies, to our knowledge, have addressed the longer-term absolute risk of CIN3 or worse among women with a persistent HPV infection (11). Previously, we found that being positive twice for high-risk HPV infection measured by HC2 tests (ie, persistence of a prevalently detected high-risk HPV infection) 2 years apart in women who initially had normal cytology was a substantially better predictor of risk for subsequent CIN3 or worse compared with one positive test during an 11-year follow-up (9). Here we have updated this finding with more than two additional years of follow-up. Although our results indicate that persistence of prevalently detected high-risk HPV DNA positivity (by HC2) can be used to stratify women into different categories of risk for high-grade CIN, this study also showed that even one newly detected HPV16 infection was associated with virtually the same absolute risk of CIN3 or worse as were two positive high-risk HC2 tests. This finding points to the relative value of HPV genotyping for defining individual risk compared with HPV DNA detection tests that are based on a cocktail of probes and thus cannot differentiate the importance of specific HPV types with regard to the risk for cervical cancer. This result is somewhat in contrast to those of Koshiol et al. (18), who reported that the persistence of specific HPV types was not more strongly associated with the risk of CIN3 or worse compared with repeated positive tests for high-risk HPV without type distinction. The apparent discrepancy between these findings could partly be explained by the fact that the women in this study were younger than those in most other studies, including, possibly, those in the study of Koshiol et al., and younger women are more likely than older women to have a prevalent transient HPV infection detected, to clear the infection, and to acquire new infections. Consequently, HPV type–specific persistence may be a better risk stratifier (compared with persistence of high-risk HPV infection measured by the HC2 test) in younger women than in older women because a higher proportion of older women who test positive twice for high-risk HPV by HC2 (persistence of prevalently detected HPV infection) will, in fact, have persistence of a specific HPV type. This hypothesis is supported by findings of a recent study from Costa Rica (11) that was similar to this study.

We also examined the importance of the individual HPV types by looking at the combined picture of prevalence, the tendency of various specific high-risk HPV types to persist, and progression to CIN3 or worse given persistence. We found that the phylogenetic group predicted both tendency to persist and carcinogenic potential because species in the alpha 9 group were more likely to persist and to progress when they persisted compared with those in the alpha 7 or alpha 5 and 6 groups. Again, HPV16 had the highest rates of prevalence and persistence and the greatest probability of leading to progression when it persisted. The estimated probability of developing CIN3 or worse at 12 years was 47.4% (95% CI = 34.9% to 57.5%) among cytologically normal women with a persistent HPV16 infection.

Persistence alone, however, may not be unequivocally and strictly related to carcinogenicity given that we observed a relatively high rate of persistence for HPV types that are only rarely found in cervical cancer and that the persistence of some HPV types (eg, HPV53, HPV56, HPV59, HPV66, and HPV68) was never or only rarely followed by CIN3 or worse. Given these findings, clinically useful algorithms for HPV persistence in primary cervical cancer screening are not entirely straightforward and thus difficult to define, and other issues will have to be resolved before HPV persistence can be taken into account in cervical cancer screening programs. For example, although HPV16 persistence in particular is a strong predictor of risk of high-grade cervical lesion or worse, women might not agree to return for retesting in 1–2 years to assess potential HPV persistence, and compliance with follow-up might decrease. An alternative would be to use a single detection of one or more of the most high-risk HPV types, such as HPV16 and/or HPV18 and perhaps HPV31 and HPV33 as well, and for infections with the other high-risk HPV types, a single combined test for infections could be used, given our finding that the subsequent risk for CIN3 or worse for them remains low for several years (Figure 2). It is reassuring that HPV DNA–negative women retained their low risk of CIN3 or worse for many years, suggesting that frequent screening of these women is unnecessary.

The strengths of this study include the relatively large cohort, the population-based study design, the long follow-up time, and the virtual absence of loss to follow-up because of the existence of the unique personal identification number and the complete nationwide registers used for the follow-up. This study also has some limitations. First, due to small numbers, we were unable to examine all HPV types individually as single-type infections in all analyses, which may have resulted in an overestimation of the rates of progression after persistence of some lower-risk HPV types. Second, because we measured HPV only twice, 2 years apart, we could not assess the duration of persistent infection and its role in the risk of progression, which has been suggested previously (18). The prevalently detected infections in this study had persisted for an unknown length of time. The duration of a persistent HPV infection is important to take into consideration if HPV testing is going to be used as a risk stratifier in screening, especially in screening programs with a short screening interval, because the likelihood of continued persistence increases with the length of time that the infection has already persisted and, again, is associated with the risk of progression. In addition, because we had no information on HPV variants, some of the infections that were defined as persistent might have been re-infections with the same HPV type, which would have resulted in an underestimation of the risk of CIN after HPV persistence. Finally, because we used a passive approach to follow up the women after the two active study examinations, this investigation is based on everyday clinical management rather than on management performed in a randomized clinical trial. This distinction might be important in the case of intensive screening and management of low-grade lesions because, in this case, the natural history may change, which could possibly lead to an underestimation of the occurrence of CIN3 or worse. However, as previously reported (9), cervical cancer

screening in Denmark is recommended every 3 years from the age of 23 years, and the management of low-grade lesions tends to be less aggressive than it is in countries such as Germany and the United States.

In summary, this relatively large prospective cohort study clearly demonstrates that infection with HPV16 is the most prevalent and has the greatest tendency to persist and the highest probability for progression when it persists. HPV18, HPV31, and HPV33 were the high-risk HPV types other than HPV16 that was associated with high absolute risks for progression. The main predictor of subsequent risk of CIN3 or worse was HPV16 persistence. One positive test and persistence (ie, two positive tests) for high-risk HPV types other than HPV16, HPV18, HPV31, and HPV33 were associated with low absolute risks of CIN3 or worse that lasted for years, indicating that a different follow-up algorithm is needed for women positive for these HPV types. Although this study contributes findings that may be important for designing more effective screening programs, several issues remain to be resolved before HPV persistence can be used in primary screening, including a standardized definition of persistence and a standardized follow-up algorithm that will not jeopardize compliance. Until other markers of HPV persistence or progression using a single measurement, are identified, type-specific HPV testing may be useful in stratifying risk in cervical cancer screening. Our results indicate that some of the HPV types that are currently classified as high risk show little potential for progression to high-grade lesions, even in cases of persistent infection; for example, our results for HPV66, which showed that persistence of this HPV type was not followed by any cases of CIN3 or worse, are in agreement with the recent re-assessment of the carcinogenicity of HPV types by an International Agency for Research on Cancer Working Group (19).

Finally, it should be mentioned that although there is an increased risk of developing CIN2 or worse associated with specific high-risk HPV types, most CIN2, if left untreated, will never progress to invasive cancer, and, in some women, notably younger women, these lesions can regress. This information is now of considerable importance given the demonstration of some obstetrical morbidity associated with definitive treatment of these lesions (20,21). By contrast, the risk of CIN3 progressing to cancer is substantial if left untreated (22).

This study provides the first population-based data to our knowledge on the long-term absolute risk of high-grade CIN after (prevalently detected) persistent infection with individual high-risk HPV types. Our findings may facilitate the interpretation of the results of screening trials, may be useful in the development of more specific HPV tests, and may identify issues that need to be resolved to obtain the greatest clinical value from HPV testing. Finally, these results may be of value in the development of new generations of prophylactic HPV vaccines and may influence the current classification of 13 class I carcinogenic high-risk HPV types.

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Funding

This work was supported by the National Institutes of Health (grant RO1 CA47812), the Sixth Research Framework Programme of the European Union, project INCA (LSHC-CT-2005-018704), the Mermaid project (MERMAID-2), Savværksejer Jeppe Juhl og Hustru Ovita Juhls Mindelegat,

and the Danish Cancer Society. Innogenetics, Inc (Gent, Belgium) provided INNO-LiPA v2HPV prototype assay kits for HPV testing free of charge.

Notes

S. K. Kjær received lecture fees, advisory board fees, and research grants from Merck and Sanofi Pasteur MSD (manufacturers of an HPV vaccine). C. Munk received lecture fees and travel grants from Merck and Sanofi Pasteur MSD. T. Iftner received speaker honoraria from Innogenetics, Inc, and Digene Corporation (maker of the Hybrid Capture 2 assay).

The study sponsors did not have any role in the design of the study; the collection, analysis, or interpretation of the data; the writing of the manuscript; or the decision to submit the manuscript for publication.

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