High-Risk Human Papillomavirus Is Sexually Transmitted: Evidence from a Follow-Up Study of Virgins Starting Sexual Activity (Intercourse)[1]

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Abstract

Genital human papillomavirus (HPV) infection is generally considered to be sexually transmitted. However, nonsexual spread of the virus has also been suggested. The goal of this study was to assess: (*a*) the role of sexual intercourse in the transmission of HPV; (*b*) the determinants for seroconversion; and (*c*) the correlation between HPV DNA, abnormal cervical cytology, and serological response to HPV16.

One hundred virgins and 105 monogamous women were randomly selected from a population-based cohort study in Copenhagen, Denmark, in which the women were examined twice with 2-year interval (interview, cervical swabs, Pap smear, blood samples). The presence of HPV DNA was determined by GP5+/6+ primers based HPV-PCR-EIA. HPV 16 virus-like particles (VLP) antibodies were detected by ELISA.

All of the virgins were both HPV DNA negative and seronegative to VLP16, except for one woman who was weakly HPV 6 DNA positive. Only those virgins who initiated sexual activity became HPV DNA positive and/or VLP16 positive. The most important determinant of HPV DNA acquisition was the number of partners between the two examinations. The only significant risk factor for HPV 16 VLP seroconversion among women acquiring HPV DNA was HPV type.

Our results show that sexual intercourse is important in the transmission of HPV, and that HPV 16 VLP seroconversion and the development of cervical lesions only occur after HPV transmission. Remarkably, no cervical lesions were found in HPV 16 DNA positive women who had seroconverted. Although based on small numbers, this may suggest that the development of antibodies had a protective effect.

Introduction

Infection with certain types of HPV[4] is the major cause of both cervical SILs and cervical cancer, but is also linked to several other types of cancer (*e.g.*, anal, vulvar, vaginal, and esophageal cancer; Ref. 1). The introduction of the PCR method and general primers in the HPV field has made available a specific and very sensitive test for the detection of HPV DNA (2, 3). For the detection of antibodies to HPV virions, a method has been developed for generating conformationally correct HPV 16 L1 virion protein (empty virus capsids), which when expressed in insect cells via recombinant baculoviruses, self-assembles into virus-like particles (VLPs) (4–6). These HPV 16 VLPs are used in an ELISA to detect IgG antibodies.

Genital HPV infection is generally considered to occur primarily by sexual transmission. Several studies are now consistent in their finding of the number of sexual partners as one of the most important risk factors associated with the presence of genital HPV DNA (7–10). However, nonsexual transmission can not entirely be ruled out, because studies have reported both perinatal transmission of HPV from mother to child (11, 12) and presence of HPV DNA in the foreskin from newborns (13), as well as in the oral mucosa of healthy preschool children (14). Furthermore, in one report, the detection of HPV 16 DNA on the vulva in 15% of virginal women has been considered to support the existence of a nonsexual route of transmission (15). However, in a recent study of virgins, Rylander *et al.* (16) found an HPV DNA positivity rate (HPV 6) of only 1.8% (2/107), and in two other studies, HPV DNA was not detected among 55 virgins (17) and 30 virgins (18), respectively.

To investigate the role of sexual activity (intercourse) in the transmission of genital HPV infection, we conducted the present study with the following specific aims: (*a*) to assess the role of sexual intercourse in the transmission of HPV DNA; (*b*) to identify the determinants for HPV 16 VLP seroconversion;

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[4] The abbreviations used are: HPV, human papillomavirus; OR, odds ratio; POR, prevalence OR; CI, confidence interval; EIA, enzyme immuno-assay; HR, high risk; LR, low risk; SIL, squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; VLP, virus-like particle(s).

Table 1 Number of women analyzed and prevalence of HPV DNA and HPV 16 VLP seropositivity at enrollment (first examination) and at follow-up (second examination) according to number of partners at the two visits

Lifetime no. of sex partners at: 1st exam./2nd exam.	First examination (exam.)						Second examination					
	HPV DNA			VLP 16			HPV DNA			VLP 16		
	n	No. of positives	(%)	*n*	No. of positives	(%)	*n*	No. of positives	(%)	*n*	No. of positives	(%)
A: 0 partners/0 partners	30	0	(0)	28	0	(0)	30	0	(0)	28	0	(0)
B: 0 partners/1+ partners	67	1	(1.5)	67	1	(1.5)	65	23	(35.4)	67	10	(14.9)
C: 1 partner/1 partner	78	2	(2.6)	78	7	(9.0)	78	4	(5.1)	77	7	(9.1)
D: 1 partner/2+ partners	27	4	(14.8)	27	4	(14.8)	26	9	(34.6)	26	6	(23.1)

and (*c*) to examine the correlation between HPV DNA, abnormal cervical cytology, and serological response to HPV16.

In the present paper, the expressions "virginal women" and "monogamous women" refer to women who have not experienced sexual intercourse and women who have only had one sex partner, respectively.

Material and Methods

Study Population. Our study population was recruited from women participating in an ongoing prospective cohort study on HPV and cervical neoplasia. From May 1991 to January 1993, a cohort of 11,088 women (20–29 years) was included from a randomly selected general population sample of women from Copenhagen. A detailed description of the enrollment and data collection procedure is given elsewhere (19). In a random sample of 1000 women from the cohort, we found that 15% were HPV DNA positive at enrollment. All subjects included in this study signed a written informed consent before participation. The study was approved by the Ethical Committee of Copenhagen and Frederiksberg Municipality, Denmark.

About two years later, the entire cohort was reinvited, and we included 8654 women in this second examination. At both visits, all of the women had a gynecological examination, and went through a personal interview conducted by trained interviewers (female nurses). The interview comprised questions about demographic and social factors, sexual, contraceptive, and smoking habits, reproductive factors, and previous sexually transmitted diseases. At the gynecological examination, material for HPV detection was obtained from the ecto- and endocervix using two plastic shafted cotton-tipped swabs. The swabs were placed in a tube with 2 ml of Tris-EDTA buffer and kept deep frozen at −80°C. In addition, a cervical smear was taken by means of another cotton-tipped swab (wooden spatula at the second examination) and a cytobrush. Finally, we also obtained two blood samples from each woman at both examinations.

From this cohort, we identified all of the women attending both examinations, who at entrance in the study reported to be virgins (*i.e.*, never had sexual intercourse) or to have had only one sex partner (respectively, 177 and 579 women). Among these two groups of women, we randomly chose 100 virginal women and 105 monogamous women, and from these, we identified four groups of women on the basis of the number of sexual partners as reported at the first and at the second visit: Group A, *virgins* who stayed virginal during the 2 years of follow-up ($n = 30$); Group B, *virgins* who initiated sexual activity (*i.e.*, sexual intercourse) at some point during follow-up ($n = 70$); Group C, *monogamous* women who stayed monogamous ($n = 78$); and Group D, *monogamous* women having new sex partners during follow-up ($n = 27$). Women with cervical swabs not suitable for HPV DNA detection at the first and/or at the second examination ($n = 7$) were excluded from the study. We also analyzed the women for the presence of antibodies to HPV 16 (VLP 16). The number of women analyzed for HPV DNA and antibodies to HPV 16 is shown in Table 1.

In the risk factor analyses, initial evaluation included univariate analysis, whereby the association between each variable and the outcome was measured by the POR with 95% CI. Variables were subsequently evaluated by means of multiple logistic regression analysis with simultaneous adjustment for confounding factors (20). All of the analyses were made using the SAS statistical software package (21).

HPV DNA Analysis. The cervical swabs were analyzed by the general primer GP5+/6+ mediated PCR-enzyme immunoassay method (22). Briefly, 10 μl of the crude cervical cell suspensions were added to the PCR mixture, which consists of 10 mM TRIS HCl (pH 8.3), 50 mM KCl, 3.5 mM $MgCl_2$, 1 unit of thermostable DNA polymerase (Amplitaq; Perkin Elmer Cetus, Norwalk, CT), 200 μmol of each dNTP, and 25 pmol of each primer (GP5+ and biotinylated GP6+). The mixture was incubated for 5 min at 94°C for DNA denaturation, followed by 40 cycles of amplification using a PCR processor (Biomed, Theres, Germany). Each cycle included a denaturation step to 94°C for 1 min, an annealing step to 40°C for 2 min, and a chain elongation step to 72°C for 90 s. To ensure a complete extension of the amplified DNA, the final elongation step was prolonged by 4 minutes. The biotinylated GP5+/6+ PCR products were analyzed by EIA using HPV HR and HPV LR oligococktail probes to identify 14 HR HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 6 LR HPV types (HPV 6, 11, 40, 42, 43, and 44), as previously described in detail (22). The HR and the LR positive swabs also were individually typed using specific EIAs. In addition, GP5+/6+ PCR products were analyzed for the presence of other HPV types not identified by the HR and LR EIAs; this was done as previously described (2) by gel electrophoresis, followed by Southern blot analysis under low stringency with a cocktail probe of different HPV types. Samples positive by this Southern blot analysis, but negative by both HR- and LR-EIA were classified as HPV X positive. Because the HR cocktail contains most HR types known to date, and the LR cocktail is incomplete, these HPV X most likely represents HPV LR types.

HPV 16 Antibody Analysis. IgG-specific seroreactivity to HPV 16 L1 VLPs was determined by ELISA as described previously (5) with the following modifications. Plates were coated with 300 ng of HPV 16 L1 VLPs in 50 μl of PBS. Human serum was diluted 1:10 in 0.5% milk-PBS. Sera were assayed three to five times on different days to ensure reproducibility. The absorbance (*A*) obtained for each assay was

Table 2 Risk of HPV DNA acquisition among virgins who initiated sexual activity during follow-up (group B)

	n	% positive	Crude POR	Adj. POR[a]	(95% CI)
No. of sex partners during follow-up					
1	38	(21.1)	1.0	1.0	
2	16	(44.8)	2.9	3.3	(0.8–13.5)
3+	11	(63.6)	6.7[b]	9.5	(1.8–49.2)
Difference in age between the woman and her first partner					
≤4 yr	40	(30.0)	1.0	1.0	
5–9 yr	15	(40.0)	1.6	1.1	(0.3–5.1)
≥10 yr	9	(66.7)	4.7[b]	5.6	(1.1–29.6)

[a] Variables adjusted for each other and for age.
[b] 95% CI excludes 1.0.

Table 3 Risk of HPV DNA detection at follow-up among women being monogamous at enrollment in the study (groups C and D)

	n	% positive	Crude POR	Adj. POR[a]	(95% CI)
Lifetime no. of sex partners at follow-up					
1	78	(5.1)	1.0	1.0	
2	11	(27.3)	6.9[b]	3.9	(0.6–25.3)
3+	15	(40.0)	12.3[b]	9.4	(2.1–41.3)
HPV DNA status at enrollment					
Negative	98	(10.2)	1.0	1.0	
Positive	6	(50.0)	8.8[b]	7.0	(0.9–52.9)

[a] Variables adjusted for each other and for age.
[b] 95% CI excludes 1.0.

normalized relative to the mean absorbance obtained for a control pooled human sera tested six times on the same 96-well plate. A preselected cut point for seroreactivity of sample *A*/control *A* = 1.00 was used. This cut point was derived in a previous study (23, 24). In cases in which positivity was ambiguous because of normalized ELISA values both above and below the cut point, the sample was designated positive if the majority of normalized values were above 1.00.

Cervical Cytology. All of the smears from both examinations, originally classified as abnormal or as benign cellular changes, as well as a random sample of some 100 normal smears, subsequently went through a review procedure. The smears were blindly reviewed by a pathologist (G. P. or M. E. S.), who was unaware of the original diagnosis, using the Bethesda nomenclature. Whenever a review diagnosis was different from the original one, a consensus diagnosis was agreed on between the two pathologists.

Results

The mean age at enrollment in the study was 24.2 years (SD = 3.0) in group A, 22.7 years (SD = 2.5) in group B, 24.4 years (SD = 2.6) in group C, and 23.3 years (SD = 2.7) in group D. In the two groups of women who had new sex partners during the 2 years of follow-up (groups B and D), the mean number of partners between the two visits was 1.8 partners (SD = 1.5) among virginal women initiating sexual activity and 2.1 partners (SD = 1.2) among initially monogamous women who did not stay monogamous.

Prevalence of HPV DNA and VLP Seropositivity. In Table 1, the prevalence of HPV DNA and VLP 16 seropositivity is presented. None of the virgins who stayed virginal had HPV DNA detected at enrollment or at follow-up. Likewise, all of these women were seronegative for HPV 16 VLP at both visits.

Among the virginal women who experienced sexual intercourse during follow-up, one woman had HPV DNA (weak signal) detected at enrollment, and she was also VLP 16 seropositive. At the second examination, 23 women (35.4%) were HPV DNA positive, and 10 women had antibodies to HPV 16, including the woman who was HPV DNA and VLP 16 seropositive at enrollment.

In the group of 78 women who stayed monogamous throughout the study period, 2 women (2.6%) had HPV DNA detected in their cervical swab taken at enrollment and 7 women were VLP 16 seropositive. At follow-up, 4 women (5.1%) had HPV DNA and 7 were positive for HPV 16 antibodies. The cumulative prevalence of HPV DNA (*i.e.*, HPV DNA positive at enrollment and/or at follow-up) was 7.7% (6/78) in this group.

Among 27 monogamous women who reported ≥2 sex partners at follow-up, 4 women (14.8%) were HPV DNA positive already at enrollment, and 4 women had antibodies to HPV 16. At the second examination, 9 women (34.6%) were HPV DNA positive and 6 women were seropositive (Table 1). The cumulative prevalence of HPV DNA was 37.0% (10/27) in this group of women.

Determinants for HPV DNA Acquisition. Risk factors for acquisition of HPV DNA were studied in the group of virgins who had their first intercourse in the time period between the two examinations (group B; Table 2). The number of sex partners during follow-up was a significant determinant, women with ≥3 partners having a 9.1 times increased risk (95% CI, 1.8–48.5) for acquiring HPV DNA when compared to women with one partner during follow-up. Likewise, an increasing age difference between the woman and her first sex partner was associated with the risk of HPV DNA acquisition. Compared to women with a first partner of the same age or <5 years older, women with a first partner who was ≥10 years older (range, 10–28 years) had a POR of 5.9 (95% CI, 1.1–33.8), when the number of sex partners between the two visits was taken into account. There was a weak tendency, which indicated that the first partner belonging to an older birth cohort increased the woman's risk of having HPV DNA, but this could be explained by the age difference between the woman and her first partner (data not shown). Age, smoking, oral contraceptive use and age of first intercourse were not significantly associated with the risk of becoming infected with HPV (data not shown).

Determinants for HPV DNA Positivity. Table 3 shows the risk factors for being HPV DNA positive at follow-up among women who had only had one partner at enrollment in the study (groups C and D). The most important determinant was the number of sex partners. Women who at follow-up reported to have had three or more partners had a 9.4 times higher risk (95% CI, 2.1–41.3) of being HPV positive than women with one partner. HPV DNA status at enrollment seemed also to be associated with being HPV DNA positive at follow-up. However, the association was no longer significant after adjustment. Age, smoking, and oral contraceptive use were not related to HPV DNA positivity at follow-up (data not shown).

Determinants for VLP 16 Seroconversion. We also examined the risk determinants for seroconversion among women who became HPV DNA positive (Table 4). The only independent determinant was HPV 16 DNA positivity at the second

Table 4 Risk determinants for seroconversion in women who became HPV DNA positive during follow-up

	n	% positive	Crude POR	Adj. POR[a]	(95% CI)
HPV DNA type at the 2nd examination					
Pos. (other types)	18	(27.8)	1.0	1.0	
Pos. HPV 16	12	(75.0)	7.8[b]	9.3	(1.6–54.3)

[a] Adjusted for age.
[b] 95% CI excludes 1.0.

Table 5 Correlation between HPV DNA and VLP 16 seropositivity: data[a] on virgins who initiated sexual activity during follow-up (group B)

Woman no.	First examination		Second examination		Pap smear result 1st visit/2nd visit
	HPV DNA (type)	VLP 16	HPV DNA (type)	VLP 16	
1	−[b]	−	16	pos	Normal/Normal
2	−	−	16	pos	Normal/Normal
3	−	−	16	pos	Normal/Normal
4	−	−	16	pos	Normal/Normal
5	−	−	16[c]	pos	Normal/Normal
6	−	−	16,[c] 18	pos	Normal/Normal
7	−	−	16[c]	pos	Normal/Normal
8	−	−	16, 18	−	Normal/LSIL
9	−	−	16, 39, 45	−	Normal/HSIL
10	−	−	18	−	Normal/Normal
11	−	−	31	−	Normal/Normal
12	−	−	33	−	Normal/Normal
13	−	−	56	pos	Normal/Normal
14	−	−	42, 31[c]	−	Normal/Normal
15	−	−	52	−	Normal/Normal
16	−	−	40	−	Normal/Normal
17	−	−	42, 51, 58	−	Normal/Normal
18	−	−	42, 51, 58	−	Normal/Normal
19	−	−	X	−	Normal/LSIL
20	−	−	X	−	Normal/Normal
21	−	−	X	pos	Normal/Normal
22	6[c]	pos	6[c]	pos	Normal/Normal
23	−	−	6	−	Normal/LSIL

[a] The Table includes women positive for HPV DNA and/or VLP 16 at one or both examinations. All of the other women were HPV DNA negative and VLP 16 negative at both visits ($n = 38$).
[b] −, negative; pos, positive; X, no HR HPV types present, but LR types other than 6, 11, 40, 42, 43, and 44.
[c] Weak signal.

examination, women who had become HPV 16 DNA positive being 9.3 times (95% CI, 1.6–54.3) more likely to seroconvert compared with women who became positive to other HPV types. Age affected the risk estimates for seroconversion, but age in itself was not a significant risk determinant (OR, 2.1; 95% CI, 0.3–14.2 for ≥27 years *versus* ≤26 years). If the analysis was restricted to virgins who started sexual activity during follow-up, the association between HPV 16 DNA positivity and VLP 16 seroconversion was even stronger ($POR_{age\text{-}adjust} = 18.5$; 95% CI, 2.1–165) (data not shown). Neither smoking nor use of oral contraceptives were associated with the risk of seroconversion (data not shown).

Correlation between HPV DNA Types, VLP 16 Seropositivity, and Cervical Cytology. In Table 5, the correlation between HPV DNA positivity and VLP 16 seroreactivity is shown for initially virginal women with first coital experience in the study period (group B). At the second examination, 9 women had become HPV 16 DNA positive, and of these, 7 women (77.8%) had also developed antibodies against HPV 16. In contrast, only 2 of 13 women who had acquired HPV DNA from other HPV types during follow-up were VLP 16 seropositive. In addition, one woman who had HPV 6 DNA (weak signal) detected at the second examination was VLP 16 seropositive [that woman (no. 22 in Table 5) was HPV 6 DNA positive and VLP 16 seropositive both at enrollment and at follow-up]. All of the women had a normal Pap smear at entrance in the study. At follow-up, four women who had become HPV DNA positive (nos. 8, 9, 19, and 23 in Table 5) had developed LSIL or HSIL. None of these women had detectable antibodies to HPV 16. In addition, one woman, who was HPV DNA negative and VLP 16 seronegative at both examinations, had developed LSIL (data not shown).

Table 6 Correlation between HPV DNA positivity and VLP 16 seropositivity: data[a] on women who were monogamous at enrollment (group C and D)

Woman no.	First examination		Second examination		Pap smear result 1st visit/2nd visit
	HPV DNA (type)	VLP 16	HPV DNA (type)	VLP 16	
1	−[b]	−	16	pos	Normal/Normal
2	16[c]	pos	16	pos	Normal/Normal
3	−	−	16	pos	Normal/Normal
4	−	pos	16,[c] 6[c]	pos	Normal/Normal
5	−	−	16, 56	−	Normal/LSIL
6	−	−	31	pos	Normal/LSIL
7	−	−	33	pos	Normal/Normal
8	−	−	33	pos	Normal/Normal
9	−	pos	18	−	Normal/Normal
10	18, 33	−	18	−	Normal/LSIL
11	−	−	11	−	Normal/ASCUS
12	−	−	56, 58	−	Normal/Normal
13	X	−	X	−	LSIL/Normal
14	X	−	−	−	Normal/Normal
15	16	−	−	−	ASCUS/Normal
16	45	−	−	−	Normal/LSIL
17	−	pos	−	−	Normal/Normal
18	−	pos	−	−	Normal/Normal
19	−	pos	−	−	Normal/Normal
20	−	−	−	pos	Normal/Normal
21	−	pos	−	pos	Normal/Normal
22	−	pos	−	pos	Normal/Normal
23	−	pos	−	pos	Normal/Normal
24	−	pos	−	pos	Normal/Normal
25	−	pos	−	pos	Normal/LSIL

[a] The Table includes women positive for HPV DNA and/or VLP 16 at one or both examinations. All of the other women were HPV DNA negative and VLP 16 negative at both visits ($n = 75$).
[b] −, negative; ASCUS, atypical cells of undetermined significance; pos, positive; X, no HR HPV types present, but LR types other than 6, 11, 40, 42, 43, and 44.
[c] Weak signal.

The correlation between HPV DNA positivity and VLP 16 seropositivity among women who at enrollment were monogamous (groups C and B) is shown in Table 6. Among the women who were HPV 16 DNA positive at the second examination, four of five women (80%) also had antibodies against HPV 16. In addition, seroconversion was observed in one woman who became HPV 31 DNA positive and in 2 women who became HPV 33 positive. No seropositivity was seen in women who were positive for other types of HPV DNA, either at enrollment or at the second examination. Furthermore, VLP 16 seropositivity was found at enrollment and/or at follow-up in nine women in whom no HPV DNA was detected.

Two of the monogamous women had an abnormal smear

at entrance in the study (nos. 13 and 15 in Table 6). Both were HPV DNA positive and VLP negative. At the second examination, nine women had developed cytological abnormalities. Of these, four women were HPV DNA positive at the second examination (nos. 5, 6, 10, and 11), 1 woman was HPV DNA positive at the 1st examination (no. 16), and one woman was HPV DNA negative (no. 25). Only two of these six women had antibodies to HPV 16 (nos. 6 and 25). The remaining three of the nine women had neither HPV DNA nor HPV 16 antibodies detected at any of the examinations (data not shown).

Discussion

In this longitudinal study on 97 women who were virgins at the start of the study and 105 women who only had one sex partner at enrollment, all of the virginal women were HPV DNA negative and VLP 16 seronegative, except for one woman, who was weakly HPV 6 DNA positive and seropositive (both at enrollment and at the second examination). Among these virginal women, only those who had coital experience during follow-up harbored HPV DNA at the second examination, *i.e.*, all of the virgins who stayed virginal throughout the study continued to be HPV DNA negative at follow-up. This strongly supports the idea that HPV is sexually transmitted. This is also supported by our serological results, which show that VLP 16 seroconversion among the virgins occurred only in those who initiated sexual activity (coitus) during follow-up.

The virgins in our study are significantly older (mean age at enrollment, respectively, 24.2 years (group A) and 22.7 years (group B)) than those included in most other studies, in which the mean age has been, respectively, 16.1 years (18) and 18 years (16, 17). This could imply that the virgins in the present study might be more likely to have been engaged in sexual activities (noncoital) that potentially could expose them to HPV. This may serve as an explanation for the one HPV 6 DNA positive virgin in our study. However, because we did not collect information on nonpenetrative sex, we cannot confirm this hypothesis. Another possible explanation could be that this woman wrongly claimed to be a virgin. This is, however, not very likely to be the case, as in the permissive Danish society it would tend to be embarrassing to be a virgin at the age of 24.

Among the women in our study population who had new sex partners during follow-up (groups B and D), the prevalence of HPV DNA at the last visit was, respectively, 35.4% (group B) and 34.6% (group D). This is in line with recent findings in young Swedish girls (18).

Although based on small numbers, it is interesting that already at enrollment a >5-fold statistically significant difference ($P = 0.032$) in the HPV DNA prevalence existed between the group of monogamous women who stayed monogamous during follow-up (group C; 2.6%) and the group of monogamous women who subsequently had new partners (group D; 14.8%). This may point to a difference in the choice of first partner between the two groups of women. Our finding is in agreement with the results of Burk *et al.* (9), who reported that important risk factors for genital HPV infection in young women include the sexual behavior of their male partner.

The cumulative incidence of HPV DNA detection in the two groups of monogamous women (C and D) was 7.7 and 37.0%, respectively. Of the 6 women with HPV DNA detected at the first examination, three women were also HPV DNA positive at the second examination. These results point to a high infection rate and to the transient nature of HPV infection in young women. This may also be supported by our finding of nine initially monogamous women who were seropositive at enrollment and/or at follow-up without having HPV DNA detected in the cervix. These women may previously have had HPV infection but have since cleared the virus.

The sexually transmitted nature of HPV infection is also strongly supported by the pattern of determinants for acquisition of HPV DNA, as identified in this study among initially virginal women who became sexually active during follow-up. The only strong characteristic of the women that was associated with the risk of acquiring HPV was the number of sex partners during follow-up. An increasing difference in age between the woman and her first sex partner also played a role. The latter could not be explained by a cohort effect or merely by the partner's age in itself. However, it may reflect measures of the first partner's sexual habits. Acquisition was not related to age in this study. This may be explained by the narrow age range in this study group with 75% of the women being between 22 and 26 years.

We observed a high concordance between acquisition of HPV 16 DNA and VLP 16 seroconversion. In our study, four of five initially monogamous women (80%), and seven of nine initially virginal, women (77.8%) who all acquired cervical HPV 16 DNA during follow-up, had also developed VLP 16 antibodies. In contrast, only 5% of women who were HPV DNA negative at the second examination, and 24% of women who were positive for other HPV types (non-16 types) at follow-up, had antibodies to VLP 16. These results point to a high sensitivity and specificity of the VLP ELISA method, and are very similar to the results from a recent Swedish study (25). These findings are also reflected in the fact that the only significant determinant for seroconversion in women who became HPV DNA positive between the two examinations was HPV type (POR, 9.3; 95% CI, 1.6–54.3 for HPV 16 positivity *versus* positivity to other HPV types), this being even clearer when restricted to virgins initiating sexual activity during follow-up (POR, 18.5; 95% CI, 2.1–165). Thus, VLP 16 serology appears to be particularly specific for HPV 16. However, seropositivity was not always stable. Of the 11 seropositive monogamous at enrollment, 4 (30%) were seronegative at follow-up. All four of the women were HPV 16 DNA negative at both examinations, possibly suggesting that transient infection results in transient seroconversion.

In the present study, we found that 11 women of 15 who had SIL detected were HPV DNA positive at the same examination (10 women) or at the previous examination (1 woman). However, most of the HPV infections did not have cytomorphological manifestations, which is what could be expected in a young, sexually active population with a high frequency of new, transient infections.

It is noteworthy, that among the 15 women who were HPV 16 DNA positive, SIL was diagnosed exclusively in the 4 women who had no antibodies against VLP 16 (nos. 8 and 9 in Table 5, and nos. 5 and 15 in Table 6). This may indicate that women with no immunological response or a delayed response to HPV 16 infection are less protected against the clinical manifestations of the infection.

In conclusion, our study strongly supports the importance of sexual intercourse for the transmission of HPV infection, as only virgins who initiated sexual activity (had intercourse) became HPV DNA positive and/or developed HPV 16 antibodies. It also demonstrates a high HPV DNA acquisition rate among young, sexually active women as well as the transient nature of the infection. The strong correlation between VLP 16 seroconversion and particularly HPV 16 DNA acquisition points to the type-specificity of the serological method.

We find that among virgins who became HPV 16 DNA

positive, only those who did not seroconvert developed abnormal cervical cytology. These results might have important implications for future prophylactic HPV vaccination.

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