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Acquisition of High-Risk Human Papillomavirus Infections and Pap Smear Abnormalities among Women in the New Independent States of the Former Soviet Union

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The rates of acquisition and the times of incident high-risk (HR) human papillomavirus (HPV) infections and Pap smear abnormalities and their predictive factors were analyzed in women participating in a multicenter screening study in three countries of the New Independent States of the former Soviet Union. The 423 patients were prospectively monitored for a mean of 21.6 months. At the baseline, 118 women were HR HPV DNA negative (Hybrid Capture II assay) and Pap smear negative (group 1), 184 were HPV DNA positive and Pap smear negative (group 2), and 121 were HPV DNA negative and Pap smear positive (group 3). The time to the acquisition of an incident abnormal Pap smear (19.4 months) was significantly longer in group 1 than in group 2 (9.2 months) ($P = 0.0001$). The times of acquisition of incident HR HPV infection were 16.6 and 11.0 months in group 1 and group 3, respectively ($P = 0.006$). The monthly rates of acquisition of incident HR HPV infections were very similar in group 1 (1.0%) and group 3 (0.8%), whereas the rate of acquisition of an abnormal Pap smear was significantly higher in group 2 (3.1%) than in group 1 (1.5%) ($P = 0.0001$). The acquisition of HR HPV infection (but not a positive Pap smear result) was significantly ($P = 0.0001$) age dependent. The only significant independent ($P = 0.001$) predictor of the incidence of an abnormal Pap smear result was a high HR HPV load of >20 relative light units/control value (CO) (rate ratio, 2.050; 95% confidence interval, 1.343 to 3.129). Independent predictors of incident HR HPV infection were patient category (a sexually transmitted disease) and ever having been pregnant. The time of acquisition of HR HPV infection was 3 months shorter than that of an abnormal Pap smear. At the baseline the high load of a particular HR HPV type is the single most important predictor of an incident Pap smear abnormality, whereas young age and having a sexually transmitted disease predict incident HR HPV infections.

The accumulated data from prospective follow-up studies suggest that the natural history of clinical human papillomavirus (HPV) infections of the uterine cervix is basically identical to that of cervical intraepithelial neoplasia (CIN) lesions, with (i) progression, (ii) persistence, and (iii) regression as the main outcome measures (7, 13, 23). However, special features of the natural history of HPV infections seem to be intimately linked to different risks of the development of cervical cancer (7, 22, 30, 31). These include the presence of latent and subclinical HPV infections and the propensity of the virus to remain persistent (latent) for prolonged periods or to become reactivated or for the infection to undergo spontaneous resolution (3, 7, 22, 30, 31).

This HPV involvement forms the biological basis of additional patterns recently described in studies of the natural history of CIN lesions, called early regression, fluctuation, and late regression (3, 20, 21, 23, 24). It seems obvious that the HPV type, viral load, the acquisition of new (incident) infections, as well as clearance of the virus are salient features of the natural history of cervical HPV infections (2, 14, 21, 22, 24, 30, 31); but data on their role as risk factors in cervical carcinogenesis have only recently emerged (1, 4). Thus, the first few studies that have addressed the mechanisms of viral clearance reported conflicting findings (12, 16), but these were sorted out in part in our recent study (S. Syrjänen, I. P. Shabalova, N.

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TABLE 1. Acquisition of abnormal PAP smear result, HR HPV infection, both, or neither in relation to the status of the women at the baseline

Status at baseline	No. (%) of women with the following acquisition event[a]:				
	Status not changed	Abnormal Pap	HR HPV	Both HR HPV and Pap	Total
Pap smear negative, HPV DNA negative	72 (61.0)	22 (18.6)	12 (10.2)	12 (10.2)	118
Pap smear negative, HPV DNA positive	73 (39.7)	111 (60.3)[b]			184
Pap smear positive, HPV DNA negative	110 (90.9)		11 (9.1)		121
Total	255 (60.3)	133 (31.4)	23 (5.4)	12 (2.8)	423 (100)

[a] The first acquisition event during follow-up. Pap, Pap smear result; HR HPV, incident HR HPV infection.
[b] $P = 0.0001$ by Pearson chi-square test.

Petrovichev, V. P. Kozachenko, T. Zakharova, A. Pajanidi, J. I. Podistov, G. Chemeris, L. G. Soazeva, E. V. Lipova, I. Tsidaeva, O. Ivanchenko, G. Pshepurko, S. Zakharenko, R. Nerovjna, L. B. Kljukina, O. A. Erokhina, M. F. Branovskaja, M. Nikitina, V. Grunberga, A. Grunberg, A. Juschenko, P. Tosi, M. Cintorino, P. Santopietro, and K. J. Syrjänen, submitted for publication). Similarly, data on the accumulation of incident HPV infections and Pap smear abnormalities are still fragmentary, and the factors predicting these events remain partly controversial (5, 6, 9, 10, 19).

We have recently completed a cohort study (the NIS Cohort Study) with more than 3,000 women in the three New Independent States (NIS) of the former Soviet Union (Russia, Belarus, Latvia) who were tested by use of optional screening tools for cervical cancer and who were subsequently monitored to study the natural history of cervical HPV infections (25, 26). In the present study, we analyzed (i) the times of acquisition of HPV infection, (ii) the monthly rates of acquisition of incident high-risk (HR) HPV infections and Pap smear abnormalities, and (iii) the predictive factors for HR HPV infections and Pap smear abnormalities (by univariate and multivariate analyses) with a cohort of 423 women. Among the women in this cohort, 118 were negative both for HR HPV DNA and by the Pap smear at the baseline, 184 were positive for HR HPV DNA but had a negative Pap smear result, and 121 were HR HPV negative but had an abnormal Pap smear result (atypical squamous cells of undetermined significance or higher).

MATERIALS AND METHODS

The subjects evaluated in the present study were derived from a subcohort of 887 women (for whom the follow-up data were available). All subjects were tested for HPV DNA and underwent a Pap smear by the protocol described above, and follow-up data from at least two (and up to seven) subsequent clinical visits were available. Of these 887 women, 423 were negative either for HR HPV DNA or by the Pap smear (or both) at the baseline and comprised the cohort for this analysis of incident events. The mean age of these women was 31.5 years (age range, 16 to 76 years), and the mean time of monitoring was 17.01 months (range, 1.7 to 41.5 months). Of the 423 women, 118 were negative for HR HPV DNA and had a normal Pap smear result at the baseline (group 1), 184 were positive for HR HPV DNA but had a negative Pap smear result (group 2), and 121 were HR HPV DNA negative but had an abnormal Pap smear result (ASC or higher) (group 3). Four women who had biopsy-confirmed CIN type III lesions in their first biopsy specimen were excluded and were treated before follow-up. In all subsequent analyses, the three groups (group 1, $n = 118$; group 2, $n = 184$; group 3, $n = 121$) were compared to each other for assessment of the factors that predict the accumulation of incident HR HPV infections and Pap smear abnormalities.

Determination of incident cases. The sample for the Hybrid Capture II (HCII) test was taken from the cervix by use of the HCII sampling kit (Digene, Silver Spring, Md.). All samples were delivered to Turku, Finland, and tested within 2 weeks to remain within the manufacturer-guaranteed test validity period of 14 days from the time of sampling. The test was performed according to the provider's instructions by using probe panel B, which detects HR HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. A relative light unit (RLU)/CO value of 1 pg/ml (approximately 8,000 copies of HPV DNA/test) was used as the cutoff for a positive test result (25).

Statistical analyses. Statistical analyses were performed by using the SPSS for Windows program package (version 11.5). Frequency tables were analyzed by the chi-square test. Pearson's *R* value or the likelihood ratio was used to assess the significance of the correlation between the categorical variables in univariate analysis. The odds ratio (OR) with the 95% confidence interval (CI) was calculated when appropriate. Differences in the means of all the continuous variables between the three groups were analyzed by either nonparametric tests (K-independent samples *t* test; Kruskall-Wallis method) or analysis of variance, when applicable. Acquisition times and monthly rates of acquisition of HPV infection and Pap smear abnormalities were compared by using nonparametric tests (Mann-Whitney U test). The McNemar test was used for paired variables (acquisition of HPV infection and Pap smear positivity) when applicable (for group 1).

Univariate survival analysis for the outcome measures (acquisition of HPV infection and/or Pap smear positivity) was based on life-table techniques (Kaplan-Meier method). All variables shown to be significant in the univariate analyses were entered into the multivariate model, run by use of Cox's proportional hazards model in a backward stepwise manner with the log-likelihood ratio statistic. In all analyses, *P* values less than 0.05 were considered significant.

RESULTS

Table 1 depicts the acquisition events (abnormal Pap smear result, infection HR HPV, both, or neither) related to the status of the women at the baseline. The probability of acquiring an abnormal Pap smear result was significantly ($P = 0.0001$) associated with the presence of HR HPV DNA at the baseline: 111 of 184 (60.3%) of the women in group 2 and 22 of 118 (18.6%) of the women in group 1 (OR, 6.63; 95% CI, 3.83 to 11.49). On the other hand, an abnormal Pap smear result at the baseline did not increase the rates of acquisition of incident HR HPV, which were identical for the women in group 1 and the Pap women in group 3: 10.2 and 9.1%, respectively ($P = 0.475$).

The times of acquisition of an incident abnormal Pap smear results for women in group 1 and group 2 were statistically significantly different: 19.4 months (range, 0.83 to 34.07) and 9.2 months (range 0.9 to 34.5 months), respectively ($P = 0.00001$, Mann-Whitney U test). This includes the 12 women for whom an incident abnormal Pap smear result was associated with an incident HR HPV infection. The times of acquisition of incident HR HPV infection in groups 1 and 2 were 16.6 months (range, 11.5 to 31.0 months) and 11.0 months (0.8

TABLE 2. Monthly rates of acquisition of abnormal Pap smear results and incident HR HPV infection in the three groups

Incident event[a]	No. of women with incident event/ woman-month at risk (%)[b]		
	Group 1	Group 2	Group 3
Pap	34/2.281 (1.49)	111/3.597 (3.08)	
HR HPV	24/2.281 (1.05)		11/1352 (0.81)
PAP and HR HPV Group 2 and 3	12/2.281 (0.52)		

[a] Pap, Pap smear abnormality; HR HPV, HR HPV infection.
[b] *P* values are 0.0001, 0.477, 0.186, and 0.0001 for differences between groups 1 and 2 for an abnormal Pap smear result, between groups 1 and 3 for HR HPV infection, for patients in group 1 who acquired abnormal Pap smear results and HR HPV infection, and between women in groups 2 and 3, respectively. *P* values were determined by the Mann-Whitney U test.

to 34.6 months), respectively ($P = 0.006$). Thus, in the women in group 1, it took some 3 months longer to acquire an abnormal Pap smear result than an incident HR HPV infection (19.4 and 16.6 months, respectively) ($P = 0.187$). On the other hand, the rate of acquisition of an incident abnormal Pap smear result in women in group 2 was more rapid than the rate of acquisition of an incident HR HPV infection in women in group 3 (9.2 and 11.0 months, respectively) ($P = 0.355$). For the whole series, it took longer to acquire incident HR HPV infection than an incident abnormal Pap smear result (14.8 and 11.6 months, respectively) ($P = 0.009$) because of the rapid accumulation of Pap smear abnormalities in group 2 (HPV positive/Pap smear negative).

Table 2 shows the monthly rates of acquisition of abnormal Pap smear results and incident HR HPV infections in the three groups. The monthly rates of acquisition of incident HR HPV infection were very similar for group 1 and group 3, whereas the rate of acquisition of an abnormal Pap smear result was more than twice as high in group 2 ($P = 0.0001$, Mann-Whitney U test) as in group 1. There was no difference in the monthly rates of accumulation of abnormal Pap smear results and HR HPV infection among the women in group 1 ($P = 0.186$), whereas the difference between group 2 and group 3 was highly significant ($P = 0.0001$) due to the high rate of accumulation of incident Pap smear abnormalities in group 2.

In life-table analyses, the cumulative incidence of abnormal Pap smear results was significantly higher in women in group 2 than in women in group 1 (Fig. 1) ($P = 0.0014$), whereas no difference in the rates of accumulation of incident HR HPV infections could be observed between group 1 and group 3 (Fig. 2). Compared with the accumulation of incident HPV infection or Pap smear abnormalities as optional events (which reached rates of 30 to 40% at 24 months), the accumulation of a coexistent abnormal Pap smear result and incident HR HPV infection by the same women was significantly slower, reaching only 10% at 24 months of follow-up (life-table not shown).

We next compared the three groups of women with different patterns of acquisition (HPV infection, abnormal Pap smear result, or both) to the women whose baseline status did not change (i.e., no incident HR HPV infection or abnormal Pap smear result) during the follow-up by performing a univariate analysis with all the data collected on the questionnaire as well as the pertinent Pap smear and HPV DNA detection data. Table 3 summarizes the factors that proved to be significant in distinguishing the women with these four outcomes. The most significant factors were age, time of follow-up, patient category, and HR HPV load.

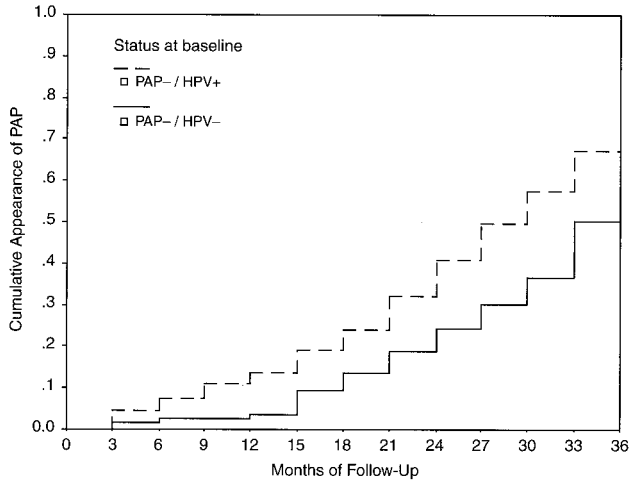

FIG. 1. Cumulative rates of acquisition of incident abnormal Pap smears (overall comparison statistic, 10.227; $P = 0.0014$). PAP−, normal Pap smear result; HPV+, HPV DNA positive; HPV−, HPV DNA negative.

To demonstrate the age dependency of incident HR HPV infection and an abnormal Pap smear result, we performed univariate (Kaplan-Meier) survival analysis for both events. While no age dependency could be demonstrated for incident Pap smear abnormalities ($P = 0.1811$) (life table not shown), the acquisition of incident HR HPV infections was significantly ($P = 0.0001$) age dependent (Fig. 3).

The significant predictors by univariate analysis (Table 3) were entered into the multivariate survival (Cox) analysis by using the backward likelihood ratio approach. The only independent predictor of the incident abnormal Pap smear result (in both HPV DNA-negative and Pap-smear negative women and HPV DNA-positive and Pap smear-negative women) was

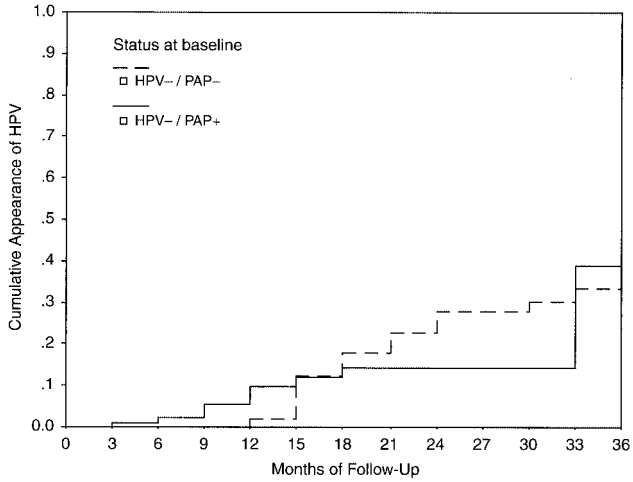

FIG. 2. Cumulative rates of acquisition of incident HR HPV infections (overall comparison statistic, 0.050; $P = 0.8238$). PAP−, normal Pap smear result; HPV−, HPV DNA negative; PAP+, abnormal Pap smear result.

TABLE 3. Factors distinguishing the women with four different acquisition outcomes in univariate analysis

Variable	Value for group				Significance (*P* value)
	Status not changed	Acquired abnormal Pap smear result	Acquired HR HPV infection	Acquired both HR HPV infection and abnormal Pap smear result	
Age (yr)[d]	32.8 (31.5–34.2)	30.0 (28.4–31.6)	27.8 (23.9–31.8)	25.2 (18.7–31.7)	0.002[a]
Time of follow-up (mo)[d]	14.2 (12.9–15.6)	22.4 (20.4–24.3)	16.9 (12.8–21.0)	17.5 (13.9–21.2)	0.0001[a]
HPV load (RLU/CO)[d]	54.2 (23.6–84.7)	209.9 (135–184)	0.31 (0.19–0.42)	0.18 (14–0.23)	0.0001[a]
Patient category[b,e]					0.0001[a]
STD	62 (24.3)	40 (30.1)	10 (43.5)	9 (75.0)	
GYN	34 (13.3)	35 (26.3)	3 (13.0)		
SCR	159 (62.4)	58 (43.6)	10 (43.5)	3 (25.0)	
Never pregnant[e]	56 (23.1)	42 (35.6)	11 (47.8)	5 (41.7)	0.01[c]
Ever had abortion[e]	140 (59.1)	46 (40.0)	11 (50.0)	5 (45.5)	0.009[c]
Ever had CIN[e]	17 (9.3)	3 (3.0)	0 (0.0)	2 (20.0)	0.027[c]
Never taken Pap test[e]	109 (54.2)	66 (62.3)	13 (72.2)	9 (90.0)	0.007[c]

[a] Kruskal-Wallis (independent-samples *t* test).
[b] GYN, gynecological health problems; SCR, screening patients.
[c] Chi-square test (Pearson's *R* value).
[d] Values in parentheses are 95% CIs.
[e] Values are numbers of women. Values in parentheses are percentages.

the high viral load. The discriminative power was highest with a cutoff level of 20 RLUs/CO (OR, 2.050; 95% CI, 1.343 to 3.129; $P = 0.001$) but lost its significance when the cutoff was raised or lowered. Viral load was not confounded by age (as a continuous variable) but maintained its independent predictive power.

Of the factors predicting incident HR HPV infection (in HPV-negative and Pap smear-negative women and HPV-negative and Pap smear-positive women), the patient category and ever having been pregnant were the only independent predictors, with *P* values of 0.0001 and 0.013, respectively. The crude rate ratio (RR) for the acquisition of incident HR HPV infection for patients who had had sexually transmitted diseases (STDs) was 10.177 (95% CI, 3.322 to 31.178) and remained significant even after adjustment for age (RR, 3.927; 95% CI, 1.193 to 12.921). The age-adjusted negative predictive power of ever having been pregnant was even more significant ($P = 0.005$; RR, 0.255; 95% CI, 0.098 to 0.664), suggesting that previous pregnancy is a significant protective factor for the acquisition of incident HR HPV infection.

DISCUSSION

The data on the risk factors that predispose women to the acquisition of incident infections and the factors that favor the clearance of prevalent HPV infections are emerging only gradually (5, 6, 9, 10, 19, 27, 28). In our study, the cumulative incidence of HR HPV infections was not significantly increased by an abnormal Pap smear result at the baseline, with only 9.1% of women with an abnormal Pap smear result at the baseline developing incident HR HPV infection. The value is practically identical to that (10.2%) for women who were Pap smear negative at the baseline and is consonant with some recent data (15). This is another indication that not all Pap smear abnormalities are related to HPV infection or that the abnormalities might be linked to the low-risk HPV types for which analyses were not performed in this study. Another feasible explanation might be that in these HR HPV-negative and Pap smear-positive women, HPV was already cleared at the time of the clinical visit and, thus, was unlikely to reappear during the follow-up. It is also well established that the panel of the HCII test does not contain all known HR HPV types, providing a minor possibility that some of these women might truly have been HPV positive at the baseline. Due to the wide coverage of the most prevalent HPV types by the HCII test, however, this possibility seems negligible and of no practical significance to the results of the study.

On the other hand, the accumulation of incident Pap smear abnormalities was significantly associated with the detection of HR HPV DNA at the baseline (Table 1). This confirms the observations previously reported by Liaw et al. (9), Rozendaal et al. (15), Moscicki et al. (10, 11), Kjaer et al. (8), and Schlecht et al. (17), all of whom could link HR HPV types with the development of incident low-grade squamous intraepithelial lesion abnormalities in the Pap smears. These data strongly suggest that the detection of HR HPV DNA at the baseline is a significant risk factor for the acquisition of Pap smear abnormalities during a relatively short follow-up, thus predisposing these woman to the development of high-grade CIN and cervical cancer (5, 7, 22, 23, 30, 31).

The factors that predict incident HPV infections have been addressed in some recent studies, but those that predict incident Pap smear abnormalities have been addressed in only a few studies (2, 6, 10, 11, 19, 27). Sellors et al. (19) and Deacon et al. (2) reported that the median numbers of sexual partners in the past year and over a lifetime are risk factors for incident

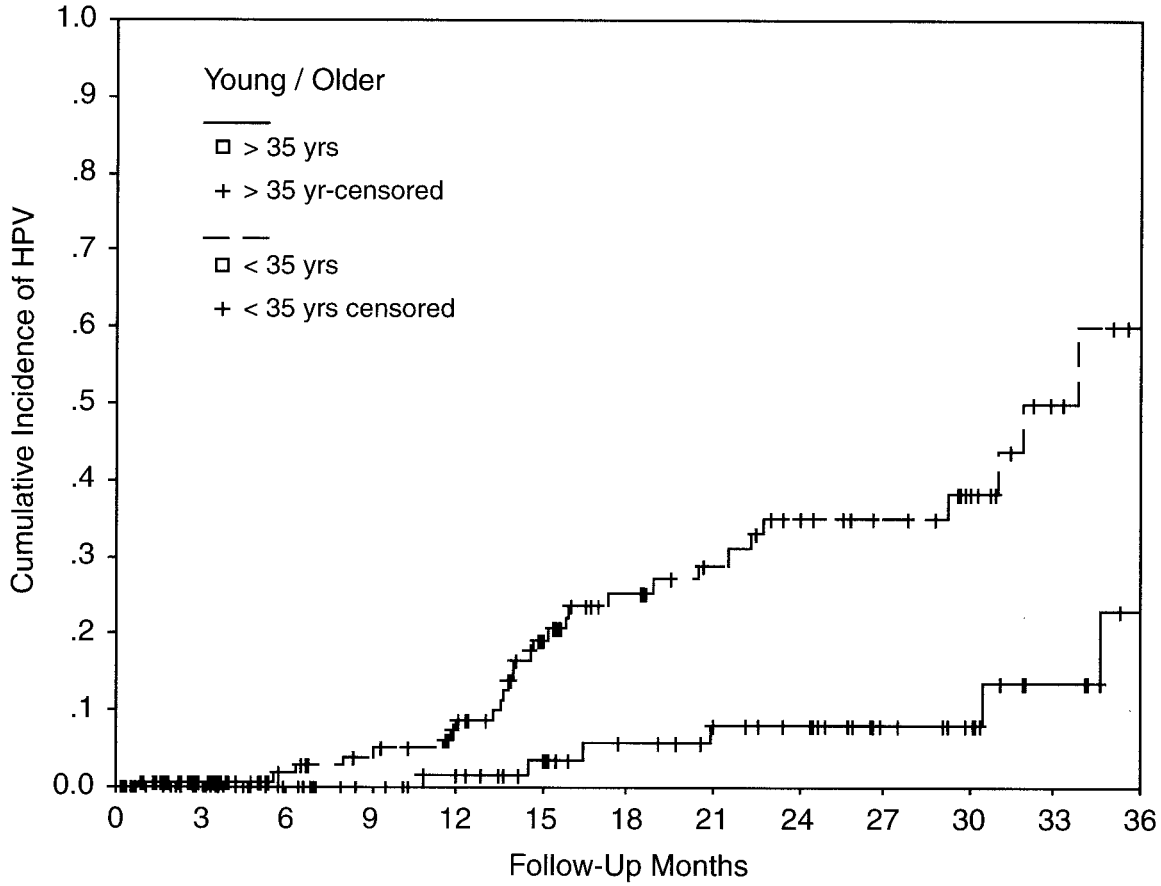

FIG. 3. Cumulative rates of acquisition of incident HR HPV infections in women younger and older than 35 years of age (by Kaplan-Meier analysis, log-rank = 14.63 and $P = 0.0001$).

HPV infections. In another study, smoking, oral contraceptive use, and a new male sexual partner were predictive of incident HPV infection (27). Moscicki et al. (11) showed that HPV infection of less than 3 years' duration and daily cigarette smoking are risk factors for incident low-grade squamous epithelial lesion in a multivariable model. Recently, Schlecht et al. (18) established the clear-cut association between the HR HPV DNA copy number and the development of incident squamous intraepithelial lesion in the Pap smear.

In the present analysis, the acquisition of an abnormal Pap smear result was not significantly age related, but women younger than 35 years of age accumulated Pap smear abnormalities at the same frequency as women older than 35 years of age ($P = 0.1811$). This is in sharp contrast to the acquisition of incident HR HPV infection, which is significantly age dependent. By univariate survival analysis, women younger than 35 years of age accumulated incident HPV infections significantly ($P = 0.0001$) more frequently than their older counterparts (Fig. 3). The acquisition rates were highest (3.7% per month) among women younger than age 20 years, which is in agreement with the recent data of Sellors et al. (19). This indicates that the rate of acquisition of incident HR HPV infection is different from the rate of clearance of these viruses, because we (Syrjänen et al., submitted) and others (5) have recently shown that the clearance of HR HPV is not age dependent but occurs at relatively constant rate across all age groups (Syrjänen et al., submitted). Young women acquire HPV infections at a significantly higher rate than older women; and because the viral clearance rate seems to be practically constant across the age groups (5; Syrjänen et al., submitted), the balance between the incident cases and the cleared cases shifts in favor of the latter in certain age groups (Syrjänen et al., submitted), explaining the progressively declining prevalence rates in older women (7, 22, 23, 30, 31). Furthermore, these different age dependencies of incident HR HPV infection and an abnormal Pap smear result are also consistent with the view that a substantial number of Pap smear abnormalities are not related to HPV infections, indicating that testing of women by the Pap smear and for HPV DNA does not necessarily screen for the same pathological condition (25).

Another significant factor distinguishing these four groups (Table 3) is the category in which they were included in the study. An unchanged status is a typical pattern for women examined as a part of routine screening, whereas acquisition of both HPV infection and abnormal Pap smear results (or either) is characteristic of patients with STDs. Previous pregnancies were typical among those whose Pap smear or HPV infection status had not changed, as were previous abortions (59.1%). Importantly, patient category and ever having been pregnant remained independent predictors of incident HR

HPV infections by multivariate analysis as well. Among the patients in this cohort, the presence of an STD can be regarded as a surrogate marker for promiscuous sexual behavior (26), although we could not relate the incident HR HPV infection and abnormal Pap smear results directly to the number of recent, current, or casual sexual partners, as some other studies have done (2, 19, 27). Similarly, a history of pregnancy is another surrogate marker for sexual behavior and reflects a stable or a nonstable partnership (26). Never having had a Pap smear was typical of women who acquired both HPV infections and abnormal Pap smear results, or either of these, compared with the group whose status had not changed (Table 3).

There was a highly significant difference in the viral loads between those women who acquired incident abnormal Pap smear results and those who did not (Table 3). The viral load proved to be the only independent predictor of incident Pap smear abnormalities by multivariate analysis. The discriminative power was highest with a cutoff level 20 RLUs/CO but lost its significance when the cutoff was raised or lowered. This is in agreement with a recent report of Schlecht et al. (18), who confirmed that the viral burden appears to have an independent effect on incident SIL. Together with the data from the univariate analysis, this suggests that both the presence of HR HPV DNA at the baseline and a high viral load are strong risk factors for incident abnormal Pap smear results during monitoring. Thus, assessment of the viral load might be helpful in identifying women at risk for high-grade CIN.

Among women who were HPV DNA negative and Pap smear negative at the baseline, the cumulative probability of incident HR HPV infection at 24 months is close to 30% and that of incident Pap smear abnormalities reaches 40% (Fig. 1 and 2). These values are comparable to those reported in some recent studies. Giuliano et al. (6) calculated a cumulative probability of incident HR HPV infection of 32% during a 12-month follow-up, while Winer et al. (27) reported a cumulative incidence of 32.3% at 24 months; these values are almost identical to those that we obtained in the present study. When several HR types are considered, all values for risk based on testing by HCII were clearly higher compared with the incidence of a specific type, e.g., prototype HPV type 16, which was 12.6% in 5 years (29). Our values are also surprisingly similar to those for a cohort of 1,075 HPV-negative and Pap smear-negative women monitored by Woodman et al. (28), who calculated a cumulative risk of 44% for any HPV infection at 3 years (Fig. 2).

For the whole cohort, it took longer to acquire HR HPV infection than an abnormal Pap smear result (14.8 and 11.6 months, respectively). This is evident from the life tables (Fig. 1 and 2), in which the survival plots for women with Pap smear abnormalities are higher than those for women with HR HPV infection. This difference, however, is due to the extremely rapid accumulation of incident Pap smear abnormalities in group 2. This higher rate of incident Pap smear abnormalities compared with the rate of HR HPV infection is another indication that a nonnegligible proportion of Pap smear abnormalities are due to causes unrelated to HPV infection. Importantly, however, in women who were HPV negative and Pap smear negative at the baseline (group 1), the time of acquisition HR HPV infection is shorter than that of an abnormal Pap smear result (16.6 and 19.4 months, respectively), indicating that HR HPV infection precedes an abnormal Pap smear result by approximately 3 months. This is consonant with our recent data on the temporal relationships of HR HPV clearance and normal Pap smear results, suggesting that the disappearance of HR HPV precedes that of an abnormal Pap smear result by 1 to 2 months (Syrjänen et al., submitted). Thus, both events are temporally closely related, but a short lag period is needed for the viral event (entry or clearance) to become manifest at the tissue level (by Pap smear or biopsy).

The times of acquisition and the monthly incidence rates of HR HPV infections are not yet generally available. Franco et al. (5) calculated a monthly incidence rate of 1.3% for new infections, resulting in 38% cumulative HPV positivity after 18 months. This is very close to the monthly incidence rates for HR HPV infection in the present study: 1.05 and 0.81% in group 1 and group 3, respectively. In another study, the incidence rate of SIL by Pap smear was 0.73 per 1,000 women-months among HPV-negative women, but it was 8.68 per 1,000 women-months among women with HPV type 16 or 18 infections that persisted over two visits (17). These values coincide with those from the present study, with a significantly higher monthly incidence rate of abnormal Pap smears in HPV-positive women at the baseline (Table 2).

Unlike incident Pap smear abnormalities, acquisition of HR HPV is clearly age dependent, being most frequent in the youngest women. The accumulation of incident abnormal Pap smears is significantly associated with the presence of HR HPV DNA at the baseline, and HPV DNA-positive women have a higher cumulative probability of an abnormal Pap smear result than HPV DNA-negative women, with this probability progressing at a higher monthly rate. By multivariate survival analysis, the only significant independent predictor of the incident abnormal Pap smear result was a high viral load, whereas independent predictors of incident HR HPV infection were patient category (patients with STDs) and ever having been pregnant (as a surrogate marker of sexual habits). At the baseline, incident HR HPV infection precedes an abnormal Pap smear result by approximately 3 months among HPV DNA- and Pap smear-negative women.

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