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Moderate Variation of the Oncogenic Potential Among High-Risk Human Papillomavirus Types in Gynecologic Patients With Cervical Abnormalities

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The oncogenic potential of human papillomavirus (HPV) infection was assessed by following the disease course in 455 patients who had had a routine diagnostic Hybrid Capture HPV test due to squamous cell abnormalities of the uterine cervix as detected by cytology and/or colposcopy. At entry, 308 patients had cytologic atypia classified as P3 by the Papanicolau classification, 168 had a positive high-risk HPV test, and 23 were infected only with low-risk HPV. The patients were followed-up using the patient registry until the endpoint of histologically diagnosed cervical intraepithelial neoplasia (CIN). High-grade CIN was diagnosed in 75 surgical biopsies. High-risk HPV infection (relative risk: 76.8 CI_{95}: 23.7–249.5), cytologic atypia (RR: 16.2 CI_{95}: 3.9–66.6), and age above 35 (RR: 1.99 CI_{95}: 1.26–3.16) were independent risk factors for high-grade CIN, while the viral load did not predict oncogenic progression ($P = 0.47$). After PCR–RFLP typing, the high-risk types were classified into groups as follows: (1) types 16 and 18, (2) types 45, 52, and 56, (3) types 31, 33, 35, 51, and 58. The relative risks of high-grade CIN were 119.1 (CI_{95}: 36.2–390.9) for group 1, 44.4 (CI_{95}: 9.8–201) for group 2, and 39.7 (CI_{95}: 10.9–144.8) for group 3, respectively. The risk ratios between the groups of high-risk types were found to differ at most by a factor of 2.98 (corrected *P* value: 0.007) indicating that the oncogenic potential varies moderately within the high-risk group of HPVs. ***J. Med. Virol. 71:585–592, 2003.*** © 2003 Wiley-Liss, Inc.

KEY WORDS: cervical intraepithelial neoplasia; hybrid capture HPV test; HPV genotype; viral load

INTRODUCTION

The etiologic role of human papillomaviruses (HPV) in the squamous cell neoplasias of the female genitalia is well established [Franco et al., 1999; Walboomers et al., 1999]. Many anogenital HPV types are known to be associated with cancer [Bosch et al., 1995; Munoz, 2000; Munoz et al., 2003] with type 16 and type 18 being the most and the second most prevalent in invasive cervical cancer worldwide [Bosch et al., 1995]. The other cancer associated types (type 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) are less commonly found in cervical cancer one by one, but together they comprise the HPVs in approximately one third of the cervical cancers. The strength of the cancer association varies with the HPV type; with high odds ratios reported for types 16, 18, 45, 52, and 59 [Munoz, 2000; Munoz et al., 2003]. For HPV type 16, the oncogenic potential further varies with intratype variants: an Asian-American variant has been reported to have a stronger cancer association than the European variants [Hildesheim et al., 2001].

Viral persistence is essential for the progression to high-grade intraepithelial neoplasia and invasive cancer [Ho et al., 1995; Nobbenhuis et al., 1999; Wallin et al., 1999] resulting in that oncogenic HPV types are detectable in the transformed squamous cells during the

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oncogenic progression. The different oncogenic HPV types are associated with high-grade intraepithelial lesions in a pattern that is similar to their association with invasive cancer [Lorincz et al., 1992; Sasagawa et al., 2001]. The combination of cytology and HPV testing can provide an improvement in sensitivity and/or specificity of cervical screening. Women with equivocal Pap smears and an oncogenic HPV infection have a strongly elevated risk of having cervical intraepithelial neoplasia (CIN) confirmed histologically [Kjellberg et al., 1998; Manos et al., 1999). Generally, sufficient diagnostic information is achieved if typing is done with a mixture of appropriate type specific probes, as it is done in the best studied commercially available HPV test, the Hybrid Capture system [Cox et al., 1995; Clavel et al., 1999; Manos et al., 1999]. However, other diagnostic test formats like reverse line blot hybridization [van den Brule et al., 2002, van Doorn et al., 2002] and array systems [Cho et al., 2003] are being introduced. These tests also provide information on the exact HPV type and whether multiple infections are present. However, the clinical and public health utility of this information is not well established and further studies are required to investigate if clinical HPV testing should aim to determine the HPV type and multiple infections.

In this hospital-based longitudinal study, we analyzed the outcome of infections by individual high-risk human papillomavirus types. A cohort of patients having had a routine diagnostic HPV test due to squamous cell abnormalities of the uterine cervix were followed until the endpoint of histologic diagnosis of CIN. Patients who did not develop CIN were censored at their last registered visit. HPV genotypes were determined from the cervical specimens and the oncogenic risk of the high-risk types was analyzed. There was a significant, but moderate variation of risk for high-grade CIN among the cancer associated HPV types.

MATERIALS AND METHODS

Study Design

This longitudinal study with prospective collection of patient data was based on the cervical screening performed at the Gynecologic Outpatient Clinic at the Medical Center of the University of Debrecen, Hungary. In Hungary, there is an organized cervical screening with voluntary participation. The primary screening involves both cytology and colposcopy. The Gynecologic Outpatient Clinic at the Medical Center acts at two levels in this screening program: first, it offers the primary screening for the local inhabitants; second, it is a regional center dealing with patients who were found to have abnormal cytology or colposcopy at the local gynecologic outpatient clinics of the region. In this regional center, HPV testing is an optional secondary screening method recommended for women where presence of an oncogenic lesion in the uterine cervix is considered to be likely by the gynecologic findings. Our cohort consisted of the 455 patients having had a cervical HPV test due to cytologic or colposcopic atypia between May 1997 and December 1999, who had no previous history of CIN or worse and made consecutive visits after the HPV testing. During the baseline test period, Digene's Hybrid Capture tube test was used to detect the low-risk and the high-risk HPV infections, separately. The follow-up data of the patients were collected from the electronic patient registry of the Medical Center. Time zero was set at the visit when the cytologic or colposcopic atypia was first detected. The diagnosis of cytologic atypia was established if the cytologic result was P3 (mild to severe dyskariosis) by Papanicolau classification. If colposcopic atypia was detected with no recent history of cytologic abnormalities, a cytologic smear was taken routinely before HPV sampling. This resulted in that several patients with mild colposcopic atypia like mosaic, punctuation, or acetowhite lesion did not have atypia in the concurrent cytologic smear. The endpoint of the follow-up was the histological diagnosis of CIN determined in cervical lesions removed by cold knife conization or large loop electrocautheric excision technique. High-grade lesions, i.e., those with histological diagnosis of CIN grade 2 or worse were considered to be of oncogenic significance. Patients not requiring surgical intervention were treated by means of conservative patient care and in the analysis were censored at the last registered visit.

Genotyping

Genotyping within the high-risk HPV group was done by RFLP analysis of the MY09–MY11 PCR products. DNA extracted from the Hybrid Capture specimens was subjected to the MY09–MY11 PCR amplification as described previously [Kónya et al., 2000], i.e., the same sample was used for both methods. At the time of therapeutic decision, the Hybrid Capture results but not the exact genotypes were known to the gynecologists. For the analysis of oncogenic potential, the high-risk HPV types were grouped as follows: HPV16 and HPV18 were classified together because these types are most commonly found in cervical cancer worldwide [Bosch et al., 1995] and also in the region where the patients of this study are from [Kónya et al., 1995]. The second group consisted of less common types (types 45, 52, 56) with reported high-risk cancer association [Lorincz et al., 1992; Munoz, 2000]. The third group consisted of the remaining oncogenic HPV types, of which types 31, 33, 35, 51, and 58 were identified among the study patients. Two additional groups were also included in the analysis: one for additional HPV types producing positive signal in Digene's high-risk HPV test and the other for the Hybrid Capture high-risk positive specimens not genotyped because of loss or unsuitability for PCR amplification.

Viral load was assessed by the strength of hybridization signal in the Hybrid Capture test and was expressed in relative light units compared to 10 pg/ml HPV16 DNA positive control (RLU/PC). According to the manufacturer's instruction, specimens with a RLU/PC ratio of one or greater were regarded positive. In the analyses,

the RLU/PC values were standardized to the DNA content of the aliquots used in the Hybrid Capture test, which ranged from 0.01 to 2.32 μg (median: 0.44 μg).

Statistical Analysis

In the different patient groups, the cumulative probability of developing CIN during the follow-up time was estimated with Kaplan–Meyer method. We carried out univariate and multivariate Cox proportional hazards regression analyses to estimate the relative risks and 95% confidence intervals (CIs) of developing CIN according to the baseline HPV and cytologic status. When a risk factor with more than two categories was analyzed, the relative risk in every risk group was related to the reference category with the lowest level of exposure. When the relative risks were calculated pairwise between multiple categories, the *P* values were corrected by the number of comparisons made. Differences in discrete type data between the patient groups was evaluated with Yates corrected chi square statistics. The differences in continuous type data like age distribution and viral load were tested with either two sample Kolgomorov–Smirnov statistics or multiple sample Kruskal–Wallis statistics.

RESULTS

Four hundred and fifty-five women were followed to investigate the occurrence of CIN in relation to HPV infection and cervical abnormalities. The age of the patients at the entry ranged from 18 to 61 (median: 32). The Hybrid Capture HPV test was performed usually at the beginning of the follow-up; only 28 patients had the HPV test more than 6 months after the first diagnosis of the cervical atypia. High-risk and low-risk HPV infection was identified in 152 and 23 patients, respectively, while the cervical specimens of 16 patients were positive in both the low-risk and the high-risk test. However, genotyping revealed multiple infections only in 5 of the 16 Hybrid Capture double positive specimens: three were infected indeed with both low-risk and high-risk types and two had double infections with high-risk types only (16 + 31 and 16 + 52, respectively). The remaining 11 double positive specimens were genotyped as infections by single high-risk types. Therefore, in the further analysis of the Hybrid Capture results the double positive patients were grouped together with the high-risk positives. This is in agreement with other studies reporting that hybrid capture high-risk positivity is associated strongly with high-grade SIL regardless of the results of the low-risk test [Schiffman et al., 1995]. The proportion of cervical atypia patients with only low-risk HPV infection (5%) was much lower than that of the high-risk positives (37%). The Hybrid Capture low-risk specimens were not genotyped systematically in this study, because it was shown previously that specimens hybridizing exclusively to the low-risk Hybrid Capture probe cocktail are infected indeed with low-risk HPV types [Kónya et al., 2000].

The data of the Hybrid Capture test, genotyping, cytologic atypia, and histologically proven CINs are shown in Table I. The histologic examination of the surgically excised biopsies revealed CIN grade 1 in 13 cases, CIN grade 2 in 31 cases, CIN grade 3 in 19 cases, carcinoma in situ in 23 cases, and microinvasive cancer in 2 cases. In the high-risk HPV infected cytologic atypia

TABLE I. Distribution of Cervical Intraepithelial Neoplasia (CIN) Cases by HPV Infection and Cytology at Entry

	Normal		P3		
Cytology	Patients	CIN (CIN2+)	Patients	CIN (CIN2+)	Proportion developing CIN2+
HPV-uninfected[a]	93	1 (0)	171	7 (3)	0.01
Low-risk HPV[a]	17	—	6	1 (1)	0.04
High-risk HPV[a]	37	2 (2)	131	77 (69)	0.41
By genotypes					
16	10		59	49 (43)	0.62
18	4	1 (1)	3	2 (2)	0.43
31	2		13	5 (4)	0.27
33	2		14	5 (5)	0.31
35	2		3	1 (1)	0.20
45			1	1 (1)	1.0
51			1	1 (1)	1.0
52	1		3	1 (1)	0.25
53			3	1 (1)	0.33
56			2	1 (1)	0.50
58			2		0
66	2		3		0
72	1				0
Multiple	6[b]	1 (1)	13[c]	5 (5)	0.26
Not typed	7		11	5 (4)	0.22

CIN2+, histologic diagnosis of grade 2 CIN or worse.
[a]As detected by Digene's Hybrid Capture tube test.
[b]Multiple infections: HPV-16/31, -16/52, -18/33, -31/56, -33/54, -45/6, -52/56.
[c]Multiple infections: HPV-16/31 (2 cases), -16/33 (2 cases), -16/6, -16/52, -16/53, -18/58, -31/52, -33/66, -45/6, -56/55, -58/6.

group, CIN was detected in 77 (58.7%) of the 131 patients and 69 (52.6%) patients had high-grade lesions, i.e., 88% of all CINs and 92% of the high-grade cases were detected in this group. In patients with negative cytology, three CIN lesions developed. One diagnosed as grade 1 at histology and was preceded also by a negative HPV test, while the other two lesions, both diagnosed as grade 2 were preceded by positive high-risk HPV tests. The rest of the CINs were detected among the cytologic atypia patients not infected with high-risk HPV: one grade 2 lesion was diagnosed 13 months after detecting low-risk (type 6) HPV infection and 7 CINs, of which 3 were high-grade, developed among HPV-negative cytologic atypia patients.

The occurrence of CIN as a function of follow-up time was analyzed in high-risk HPV infected and uninfected patients stratified by the presence or absence of cytologic atypia (Fig. 1). HPV results obtained with the Hybrid Capture test were used in this analysis. In the high-risk HPV infected cytologic atypia group, there was a high probability of having the histologic diagnosis of CIN during the 1st year of the follow-up, suggesting that in many cases there had been prevalent CIN already at the first diagnosis of the cervical atypia. Not only the rate but also the distribution of CINs was different between the high-risk HPV infected and the HPV uninfected groups. In the HPV negative cytologic atypia group, 5 of the 7 cervical intraepithelial neoplasisas including two high-grade ones were diagnosed more than 2 years after the entry. In the latter cases, the cytologic abnormalities first regressed but recurred thereafter and eventually, the lesions were excised.

The age distribution of the above patient categories was uneven: patients with cytologic atypia were older than those without (median age: 34 vs. 28, $P = 0.001$); in the cytologic atypia group, the HPV uninfected patients were older than those with high-risk HPV infection (median age: 38 vs. 31, $P < 0.001$). Among the patients without cytologic atypia, the age distribution of HPV uninfected and high-risk HPV infected patients was not significantly different (median age: 28 vs. 26, $P = 0.59$). Therefore, the effect of age on developing CIN was computed together with high-risk HPV infection and cytologic atypia in multivariate analysis (Table II), which revealed that all three factors were independent risk factors. It is of interest that increasing age proved to be a risk factor only if it was adjusted to HPV infection. The underlying reason for this finding was that the lower risk of CIN after high-risk HPV infection at younger age was balanced with higher HPV prevalence, which resulted in a CIN rate (16%) similar to that of the older patients (18%).

Of the 168 specimens found high-risk HPV positive in the Hybrid Capture test, 150 (89.2%) were genotyped by PCR–RFLP and were used to evaluate differences in oncogenic potential within the high-risk group of HPVs. The patients whose infecting type was not determined did not differ from those with known genotypes in age distribution ($P = 0.79$) and in the rate of CIN ($P = 0.28$). Genotyping revealed that co-infections with multiple

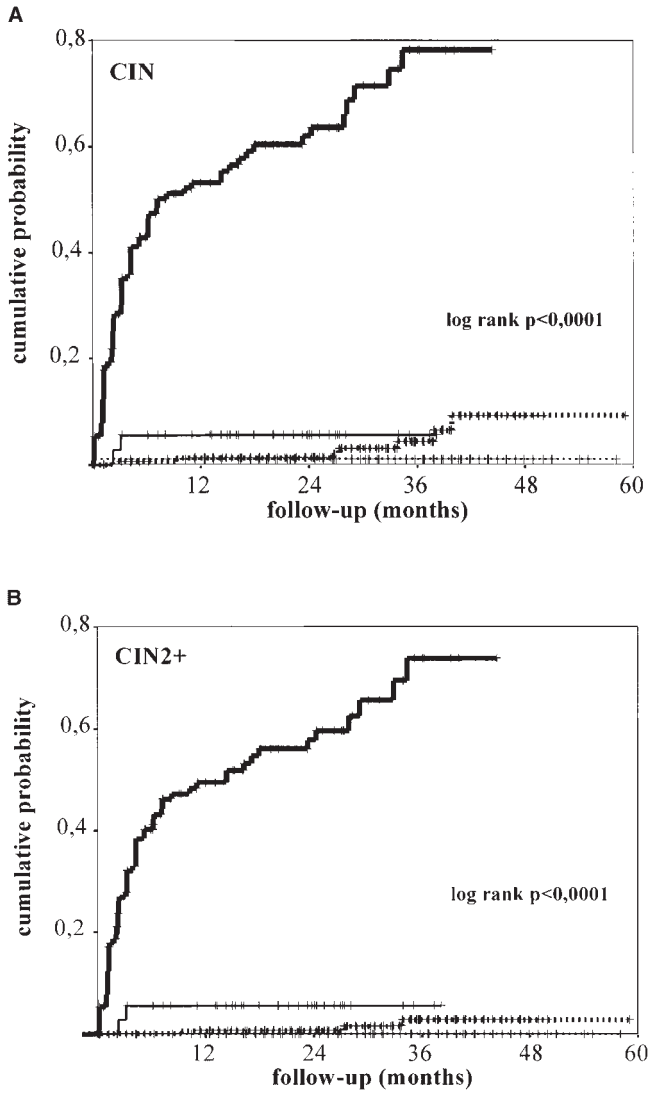

Fig. 1. Cumulative probability of developing cervical intraepithelial neoplasia (CIN) (**A**) and high-grade CIN (**B**) in patients with high-risk HPV positive cytologic atypia (thick solid line), with high-risk HPV infection but without cytologic atypia (thin solid line), with HPV negative cytologic atypia (thick dashed line), with negative HPV, and cytologic results (thin dashed line). The analysis is based on the results of the Hybrid Capture HPV test.

HPV types were present in 19 (12.7%) patients, who tended to be younger than the patients with single HPV infection (median age: 24 vs. 31, $P = 0.056$). The median viral load of the single and the multiple infections were 23.2 RLU/PC (range: 2.0–164) and 10.9 RLU/PC (range: 1.0–262), respectively ($P = 0.27$). The risk of CIN after co-infection with multiple HPV types was somewhat but not significantly less than after single high-risk infections both in univariate and multivariate analysis (Table II). The risk ratio between the multiple and the single infection for development of high-grade CIN was 0.58 (CI_{95}: 0.25–1.34). Taking both the single and the multiple infections into account, the most common types were HPV16, HPV33, HPV31, and HPV18, which were found in 78 (52%), 21 (14%), 20 (13.3%), and 9 (6%) cases, respectively. Less common high-risk types

TABLE II. Relative Risks (With 95% Confidence Intervals (CIs)) for Cervical Intraepithelial Neoplasia (CIN) According to Viral Status, Cytology, and Age

	CIN			High-grade CIN			
Risk factor	Cases/ followed patients	Crude RR	Adjusted RR[a]	Cases/ followed patients	Crude RR	Adjusted RR[a]	Follow-up (patient months)
HPV infection							
No[b]	8/264	1.0	1.0	3/264	1.0	1.0	7965
Low-risk type[b]	1/23	1.67 (0.21–13.4)	2.10 (0.26–16.9)[c]	1/23	4.42 (0.46–42.6)	5.6 (0.57–53.6)[c]	567
High-risk type[b]	79/168	30.8 (14.5–65.4)	34.0 (15.9–73.1)	71/168	66.9 (208–214.8)	76.8 (23.7–249.5)	2112
Single high-risk	68/131	35.9 (16.8–77.1)	39.1 (18.0–85.0)	61/131	76.2 (23.6–245.7)	85.6 (26.2–279.3)	1602
Multiple high-risk	6/19	16.2 (5.5–47.2)	19.3 (6.6–57.1)	6/19	39.9 (9.9– 161.0)	49.5 (12.2–201.7)	332
High-risk types							
16, 18	57/87	47.3 (22.0–102.0)	52.7 (24.2–114.8)	51/87	102.0 (31.4–331.0)	119.1 (36.2–390.9)	950
31, 33,35, 51, 58	12/43	18.2 (7.3–45.0)	17.2 (6.9–43.2)	11/43	40.4 (11.1–146.7)	39.7 (10.9–144.8)	253
45, 52, 56	4/11	15.8 (4.7–53.3)	17.8 (5.3–60.5)	4/11	38.5 (8.6–172.7)	44.4 (9.8–201.0)	575
Cross-reacting types[d]	1/9	6.17 (0.76–49.8)	8.4 (1.03–69.0)	1/9	14.7 (1.52–142.1)	21.4 (2.2–209.2)	156
Not typed	5/18	20.4 (6.5–64.0)	29.6 (9.4–93.4)	4/18	38.7 (8.5–175.6)	58.3 (12.8–266.3)	178
High-risk viral load (RLU/PC)[e]							
1–10	37/80	1.0	1.0	29/80	1.0	1.0	973
10–100	37/74	1.08 (0.68–1.70)	1.21 (0.77–1.92)	37/74	1.38 (0.85–2.25)	1.54 (0.95–2.51)	950
>100	5/14	0.73 (0.29–1.88)	0.67 (0.26–1.72)	5/14	0.95 (0.37–2.45)	0.86 (0.33–2.23)	189
		$P = 0.81$	$P = 0.90$		$P = 0.51$	$P = 0.47$	
Cytology							
Normal	3/147	1.0	1.0	2/147	1.0	1.0	4264
P3	85/308	16.5 (5.2–52.2)	12.5 (3.9–40.0)	73/308	21.0 (5.2–85.5)	16.2 (3.9–66.6)	6380
Age							
<35	49/267	1.0	1.0	41/267	1.0	1.0	5739
>=35	39/188	1.11 (0.73–1.69)	1.85 (1.20–2.85)	34/188	1.15 (0.74–1.83)	1.99 (1.26–3.16)	4905

[a]Relative risks according to either HPV typing method are adjusted to cytology and age, relative risks according to viral load are adjusted to cytology, age, and the DNA content of the aliquots used in the Hybrid Capture test, relative risks according to cytology and age are adjusted to each other plus HPV infection as detected by the HCT test.
[b]As detected by Digene's Hybrid Capture tube HCT test.
[c]Adjusted only for age but not cytology because cytologic abnormalities were uncommon in this group.
[d]Types found to cross-react with HCT high-risk probe were 53, 66, and 72.
[e]Relative light units per 10 pg/ml HPV16 DNA positive control.

(HPV35, HPV45, HPV51, HPV52, HPV56, HPV58) were present in 26 (17.3%) cases (Table I). The analysis of the patients with genotyped high-risk HPV infection revealed that HPV16 infection tended to be more common in the cytologic atypia group than in patients without cytologic atypia (55% vs. 40%, $P = 0.20$), while HPV18 was significantly less frequent in the cytologic atypia group (3.3% vs. 16.7%, $P = 0.016$). On the other hand, HPV31, HPV33 and the other high-risk types were distributed in an even manner between the cytologic atypia and normal cytology groups (P values: 1.0; 1.0; 0.66; respectively).

Due to the limited size of the study, the high-risk genotypes were grouped as mentioned above. Since infection by multiple HPV types did not have higher oncogenic risk than infection by a single type (Table II), we classified the multiple infections together with the single infections as follows: if one of the co-infecting types were HPV16 or HPV18, the multiple infection was analyzed together with the HPV 16/18 group; if one of the co-infecting types were HPV45, HPV52, HPV56 but the other co-infecting type was not HPV16 or HPV18, the multiple infection was analyzed together with the HPV 45/52/56 group; the multiple infections with co-infecting high-risk types other than above were analyzed together with the HPV 31/33/35/51/58 group. Among the patient groups classified by the different high-risk HPV types, the differences in age distribution ($P = 0.57$) and in the frequency of cytologic atypia ($P = 0.63$) were not significant.

The multivariate estimates of the relative risk revealed that all high-risk genotypes were significant risk factors for CIN (Table II). The highest risk of CIN was associated with the group of HPV16 and 18. The pairwise differences between the HPV groups (Table III) revealed that the group of HPV16 and 18 had approximately three times higher oncogenic risk than the less common cancer associated types. Very similar risks for high-grade CIN were estimated among the less common cancer associated types, i.e., the group of types 45, 52, 56 did not prove

TABLE III. Intertype Relative Risks (RR, With 95% CI) for High-Grade CIN Adjusted for Cytology and Age

	Reference category		
Tested category	Cross reacting low-risk types	Types 31, 33, 35, 51, 58	Types 45, 52, 56
Types 16, 18	5.38 (0.74–39.3)	2.98[a] (1.55–5.74)	2.74 (0.98–7.64)
Types 45, 52, 56	1.38 (0.13–14.4)	1.15 (0.36–3.67)	—
Types 31, 33, 35, 51, 58	1.42 (0.18–11.3)	—	—

Relative risks were calculated pairwise between the categories of HPV types shown in Table II.
[a]$P_{corrected} = 0.007$.

to have higher oncogenicity than the group of types 31, 33, 35, 51, 58. In nine cases, HPV type 53, 66, or 72 were identified, which can cross-hybridize with the high-risk probe cocktail of the Hhybrid Capture tube test [Kónya et al., 2000]. One HPV53 infection ended in CIN grade 2 at month 3 of the follow-up resulting in a significant risk (RR: 19.2 CI_{95}: 1.97–186) for high-grade CIN in this group of patients. The risk of high-grade CIN after infection by the cross-reacting types tended to be 1.38-5.38 times less than after infection by the established high-risk types (Table III).

The viral load was assessed by the strength of the hybridization signal (RLU/PC) measured in the Hybrid Capture test (Table II). Since high-risk HPV infection was a major risk factor, we analyzed whether or not the risk can be further refined by the RLU/PC values in patients with high-risk HPV infection. In this group, the RLU/PC values ranged from 1.0 to 262.5 (median: 10.5). This estimate of the viral load was associated with neither cytologic atypia ($P = 0.87$) nor age above 35 ($P = 0.77$) nor the different groups of high-risk types ($P = 0.70$). The viral load also did not predict the high-grade CIN (Table II). Nevertheless, all of the eight HPV-positive grade 1 lesions were associated with RLU/PC value below 10.

DISCUSSION

Definition of the risk associated with type-specific HPV positivity provides insight into the pathogenesis of cervical cancer and could possibily be used to improve screening programs. The present study focused on the interpretation of genotyping in routine gynecologic care using HPV testing as a secondary screening method. This means that HPV testing was indicated by cervical abnormalities resulting in that many, presumably prevalent, CINs were diagnosed in the 1st year of the follow-up. Due to the particular primary screening using both cytology and colposcopy, also patients with cytologic result of no oncogenic significance were followed. As expected, both high-risk HPV infection and atypical cytology were major risk factors for having CIN or worse histologic diagnoses in this study. Older age proved to be also an independent risk factor, although the strength of the association was considerably weaker than that of the high-risk HPV infection or the atypical cytology. The coexistence of high-risk HPV infection and cytologic atypia is known to provide a sensitive single point measurement for intraepithelial neoplasia [Ho et al., 1995]. Consistently, most but not all high-grade CINs were detected in the high-risk HPV positive cytologic atypia group. Nevertheless, all high-grade CINs were predicted by either cytologic atypia or high-risk HPV infection emphasizing the high sensitivity of combined cytology and HPV testing.

Beyond the detection of high-risk HPV infection, also the impacts of multiple infections, genotypes, and viral load were analyzed. The Hybrid Capture test itself indicated co-infections by low-risk and high-risk types in some cases, but most of these results could not be confirmed by genotyping. On the other hand, genotyping revealed multiple infections in some of the Hybrid Capture high-risk positve specimens. The proportion of multiple infections as determined by genotyping was similar to previous reports from Central Europe [Tachezy et al., 1999]. Multiple HPV infections did not confer any increased oncogenic risk over single high-risk infections. In fact, the point estimates of the relative risk of CIN after multiple infections were lower than after single infections. This is similar to the findings of a population-based study of cervical cancer in Costa Rica [Herrero et al., 2000]. Regarding the most prevalent high-risk HPV types of this study, the prevalence of HPV16, 33, and 31 in cytologic atypia was similar to that in invasive cervical cancer in Europe [Bosch et al., 1995], while the prevalence of HPV18 in cytologic atypia was lower than in invasive cancer, which is in agreement with other studies [Kalantari et al., 1997; Nindl et al., 1999]. HPV18 had a negative association with cytologic atypia, which is concordant with the results of Woodman et al. [2003], who concluded that the under-representation of cytologic abnormalities in HPV18 infection may understate the severity of the HPV18-mediated oncogenic progression in the cytology based prevention strategies. Indeed, one of the two high-grade CINs of this study with no recent history of cytologic atypia was HPV18 positive. The other case had a double infection with types 16 and 52.

Because of the low number of the less common types one by one, the high-risk HPV types were grouped according to the odds ratios reported in pooled hospital based case-control studies [Lorincz et al., 1992; Munoz, 2000]. A recent report of population based case-control studies [Munoz et al., 2003] found odds ratios somewhat different from those calculated from the hospital-based studies. Nevertheless, the lower confidence limits of the odds ratios in the latter study have a rank that is very similar to that of the hospital based studies. There is

only one rare type, the HPV56, whose oncogenicity was found to be high in one study [Lorincz et al., 1992], and low in the other [Munoz et al., 2003]. However, the raw data of individual genotypes and high-grade CINs (Table I) did not support a reclassification of HPV56 into a lower risk group in this study.

Consistent with the above mentioned studies, an approximately three times difference was found in the oncogenic potential between the group of types 16 and 18 and the group of less common high-risk types. The results of this study differ from the above-mentioned studies in that the point estimates of relative risks were very similar between the group of HPV45, 52, 56 and the group of other less common high-risk types. Possible reasons for this difference can be that different disease groups were examined; patients were from different geographic region, the longitudinal study design, or random fluctuation.

The oncogenic potential of the types cross-reacting with the Hybrid Capture high-risk probe was also analyzed. One of them, HPV53 used to be classified as a low-risk type [Meyer et al., 2001] but later the possibility that HPV53, HPV66, and HPV72 can be oncogenic was raised [Herrero et al., 2000; Munoz et al., 2003]. It was found that the risk of Hybrid Capture cross-reacting types is intermediate between those of high- and low-risk HPV types.

To interpret the impact viral load on the outcome of the cervical disease, it must be considered that the HPV testing was done as a secondary screening of patients selected by cytologic or colposcopic disorders. Thus, the results of this study indicated that viral load data do not refine the risk for cervical neoplasia among patients with cervical abnormalities. On the other hand, several studies compared the viral load between patients with cervical disease and HPV infected controls without cervical disease [van Duin et al., 2002; Gravitt et al., 2003; Schlecht et al., 2003]. These settings provide a good assessment on the impact of viral load data when HPV is tested already in the primary cervical screening, which will reveal also a group of HPV infected women with no cervical disease and lower viral load. This phenomenon was pointed out by Lorincz et al. who demonstrated in the Kaiser Permanente cohort that increased risk for future carcinoma in situ or worse inferred by increasing viral load is eliminated by adjustment for the baseline cytologic results [Lorincz et al., 2002]. Most studies investigating the significance of the viral load used quantitation based on PCR amplification, though only few of them provided data about high-risk HPV types other than type 16 [Gravitt et al., 2003; Schlecht et al., 2003]. PCR amplification detects much lower copy number than the Hybrid Capture test and in the recent years, also the consensus PCR methods such as that using PGMY09/11 primers have been optimized to detect a uniformly low copy number of the respective HPV genotypes [Castle et al., 2002b]. Using in either the primary [Clavel et al., 1999] or the secondary screening [Manos et al., 1999], the Hybrid Capture HPV test identifies sensitively the patients with oncogenic risk. Since PCR methods can detect the virus also in women with viral load below the detection threshold of the Hybrid Capture test, the PCR based quantitation can extend the lower tail of the viral load range by including cases who are unlikely at oncogenic risk. Thus, analyzing the impact of viral load through this extended range will reveal more definite differences in oncogenic risk than the analysis restricted to the Hybrid Capture positive, i.e., >1 RLU/PC values [Gravitt et al., 2003].

A significant but moderate variability was detected in the oncogenic potential of different HPV types. The optimal composition of an HPV-based screening test can not be determined by the present study, as it is dependent on the required longitudinal sensitivity and specificity as well as the associated costs. Also, the only definitely significant different HPV type-specific risk supported by a substantial number of observations is the elevated risk associated with HPV type 16: >60% of the HPV16 positive patients developed CIN, a considerably larger proportion than among patients infected with the group of other oncogenic types. From the diagnostic point, the low level cross-reactivity of Hybrid Capture high-risk HPV tests [Kónya et al., 2000; Castle et al., 2002a; Poljak et al., 2002] is not necessarily a disadvantage, since the cross-reacting types may also have an elevated oncogenic risk. New test formats [van Doorn et al., 2002; Cho et al., 2003] providing information on the infecting genotypes are of obvious benefit in certain cases, such as follow-up after surgical excision of CIN [Nobbenhuis et al., 2001; Elfgren et al., 2002] or detection of type specific persistence [Kjaer et al., 2002] especially in the absence of cytologic abnormalities and in HPV18 infection [Woodman et al., 2003]. The preventive vaccines under development are specific to a restricted number of types and HPV testing in the post vaccination era may also require HPV typing.

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