Multicentric Research

Factors predicting persistence of high-risk human papillomavirus (HPV) infections in women prospectively followed-up in three New Independent States (NIS)* of the former Soviet Union

S. Syrjänen[1], *DDS, Ph.D.*; **I. Shabalova**[2,3], *M.D., Ph.D., MIAC*; **N. Petrovichev**[2], *M.D., Sc.D.*; **V. Kozachenko**[2], *M.D., Sc.D.*; **T. Zakharova**[2], *M.D., Ph.D.*; **J. Pajanidi**[2], *M.D., Ph.D.*; **J. Podistov**[2], *M.D., Ph.D.*; **G. Chemeris**[2], *M.D., Ph.D.*; **L. Sozaeva**[3], *M.D., Ph.D.*; **E. Lipova**[3], *M.D., Ph.D.*; **I. Tsidaeva**[4], *M.D.*; **O. Ivanchenko**[4], *M.D.*; **A. Pshepurko**[4], *M.D.*; **S. Zakharenko**[5], *M.D.*; **R. Nerovjna**[6], *M.D.*; **L. Kljukina**[7], *M.D., Sc.D.*; **O. Erokhina**[7], *M.D.*; **M. Branovskaja**[8], *M.D., Ph.D.*; **M. Nikitina**[9], *M.D., Ph.D.*; **V. Grunberga**[9], *M.D.*; **A. Grunberg**[9], *M.D.*; **A. Juschenko**[9], *M.D.*; **M. Cintorino**[10], *M.D., Ph.D.*; **R. Santopietro**[10], *M.D., Ph.D.*; **P. Tosi**[10], *M.D., Ph.D.*; **K. Syrjänen**[10, 11], *M.D., Ph.D., FIAC*

[1]Department of Oral Pathology, Institute of Dentistry, and MediCity Research Laboratory, University of Turku, Turku, Finland; [2]N.N. Blokhin Cancer Research Centre of Russian Academy of Medical Sciences (RAMS), Moscow, Russia; [3]Russian Academy of Post-Graduate Medical Education, Moscow, Russia; [4]Novgorod Clinical Regional Hospital, Centralised Cytology Laboratory, Novgorod, Russia; [5]Novgorod Municipal Dermato-venereological Dispensary, Department of Gynaecology, Novgorod, Russia; [6]Novgorod Female Consultative Outpatient Hospital, Department of Gynaecology, Novgorod, Russia; [7]Research Institute of Oncology and Medical Radiology, Republican Centre of Clinical Cytology, Minsk, Belarus; [8]Minsk State Medical Institute, Department of Gynaecology and Obstetrics, Minsk, Belarus; [9]Latvian Cancer Centre, Department of Gynaecology, & Laboratory of Cytology, Riga, Latvia; [10]Department of Pathology, University of Siena, Siena, Italy; [11]Department of Oncology & Radiotherapy, Turku University Central Hospital, Turku (Finland)

Summary

Background: We completed an analysis of the factors predicting the persistence of high risk (HR) HPV infections in women participating in a multicenter screening trial in three NIS countries.

Methods: The 543 baseline HR HPV-positive women included in this analysis are derived from a sub-cohort of 887 women who were prospectively followed-up for a mean of 21.6 months (range: 0.5-42.9) as a part of a multi-center screening study in three NIS countries (the NIS cohort study; n = 3,187 women). Of these 543 women, 273 showed persistent HR-HPV in serial Hybrid Capture II (HCII) testing during the follow-up (Group 1), whereas 270 women cleared their infection (Group 2). These two groups were compared with their epidemiological, clinical, and virological data (HCII, PCR) to disclose the factors predicting persistent HR-HPV infection.

Results: Women with persistent HR-HPV infections were significantly younger (27.3 yrs) than those who cleared their infection (29.1 yrs) (p = 0.006), and their follow-up time was shorter; 14.1 and 21 months, respectively (p = 0.0001). Both variables were treated as confounders in the multivariate analyses. Of the 66 recorded epidemiological variables, only being a current smoker proved to be an independent predictor (OR 1.693; 95% CI 1.114-2.573; p=0.014). Baseline colposcopy, biopsy or Pap smear did not predict HPV persistence, whereas an incident or persistent abnormal Pap during the follow-up were independent predictors in a multivariate model (p = 0.005), together with the high viral load (HCII RLU/CO at 100 pg/ml cut-off), and HR HPV positive PCR test (p = 0.0001). When all significant variables were entered in the regression model, only the follow-up time (OR 0.950, 95% CI 0.924-0.976; p = 0.0001) and HR-HPV positive PCR (OR 4.169, 95% CI 1.741-9.987; p = 0.001), remained independent predictors.

Conclusions: While several factors were related to HR-HPV persistence in univariate analysis and when adjusted for age and follow-up time as confounders, the only independent predictors in the multivariate regression model were follow-up time and HR-HPV positive PCR. Clearly more data are needed on type-specific persistence and HPV integration as its predictors.

Key words: High-risk HPV; Persistent infection; Virus clearance; Predictors; Screening; Hybrid Capture II.

*The NIS Cohort Study.

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Introduction

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 CIN lesions, with a) progression, b) persistence, and c) regression as the main outcome measures [1-3]. However, HPV infections have special features in their natural history that are related to the different risks of developing cervical cancer [4-7]. It seems obvious that HPV type, viral load, acquisition of new (incident) infections as well as clearance of the virus, are salient features of the natural history of cervical HPV infections [2, 3, 7-14], but their significance in cervical carcinogenesis is incompletely understood. Accordingly, the data on the accumulation of incident HPV infections are still imperfect and the factors predicting these events are controversial [12, 15-18]. Similarly, the studies addressing the mechanisms of viral clearance have reported conflicting findings [19-21].

Recently accumulated evidence suggests, however, that persistent infections of the high-risk (HR) HPV types play a key role in the progression of CIN lesions and in the development of cervical cancer [4-11, 22-24]. During the past few years, a number of studies have analysed the host and viral factors as determinants of HR-HPV persistence [8, 13, 14, 22-26]. Comparison of these data is not straightforward, however, because of the significantly different study design, definition of virus persistence as well as the methods used for detection of HPV. Some of the factors predisposing women for HR-HPV persistence seem to be reasonably well established, however, including immunosuppression by HIV or other reasons [4, 7, 13, 27] and high viral load [8, 14, 22-24]. On the other hand, further data on the role of dietary intake [14, 26], p53 codon 72 polymorphisms [8, 15], polymorphisms of individual HPV types [8, 14, 15, 28], pregnancy [29], virus integration [5, 8-10, 14, 15] and smoking [8, 14, 30], as determinants of HR- HPV persistence are still needed [4, 5, 7].

We recently conducted a cohort study testing over 3,000 women for optional screening tools in three New Independent States (NIS) of the former Soviet Union, and almost 900 of these women were followed-up to assess the natural history of their HPV infections (the NIS Cohort study) [31, 32]. In a series of previous reports, we analysed the temporal relationships between the viral events (i.e., acquisition and clearance of HR-HPV) and the clinical disease (incident lesions, disease regression) [12, 21]. According to these data, incident HPV infections are closely age-dependent, in contrast to virus clearance which is not age-related; both events precede (by a few months) the development and disappearance of the clinical disease, respectively [12, 21, 33].

Prompted by these new data [12, 21, 33], we extended the analysis into a sub-cohort of 273 women who had persistent HR-HPV infections during the follow-up. For comparison, a series of 270 women who cleared their HR-HPV infection were included in the analysis. The purpose of the present study was to unveil the factors predisposing these women to persistent HR-HPV infections, among the data collected by an epidemiological questionnaire, clinical examination (colposcopy, Pap, biopsy), and virological analysis (Hybrid Capture II, PCR) of their samples.

Material and Methods

Study Design

The subjects of this study represent a sub-cohort of 543 women derived from a major cross-sectional/cohort study, conducted between 1998-2002 in three New Independent States (NIS) of the former Soviet Union, and comprising a cohort of 3,187 women enrolled by six outpatient clinics in Moscow, Novgorod (Russia), Minsk (Belarus) and Riga (Latvia) [31]. Three target populations of women with different risks for HPV infections were enrolled: 1) women participating in cervical cancer screening (=SCR patients); 2) those attending gynaecology outpatient clinics with different indications (=GYN patients), and 3) patients examined at sexually transmitted disease (STD) clinics (=STD patients).

The study design has been detailed in two previous papers [31, 32]. All women had three tests performed: 1) Pap smear (ARM I), 2) PCR (ARM II), and 3) Hybrid Capture II (ARM III). Women with cytological abnormality consistent with atypical squamous cells (ASC) or higher and those testing positive with Hybrid Capture II (HCII) were referred for colposcopy and biopsy confirmation [31, 32].

Follow-up

All women who presented with low-grade lesions (HPV-NCIN or HPV-CIN I) were prospectively followed-up, while high-grade lesions were promptly treated, as detailed before [31, 32]. Follow-up at 6-month intervals included examination by colposcopy, Pap smear and punch biopsy (in case of suspected progression). Cytological samples for HPV DNA testing with HCII and PCR were collected at each follow-up visit. Altogether, follow-up data were available from 887 women, among whom the two cohorts were derived for the present analysis.

Patients studied for persistent HPV infections and virus clearance

The present study analysed the factors predicting persistence of HR-HPV infections in these women, using the patients who cleared their HR-HPV during the follow-up as a comparison group. The subjects analysed for persistent HR-HPV infections comprise a series of 273 women who were HR-HPV positive at baseline, and did not clear their infection during the follow-up. Of these 273 women, 193 had also an abnormal Pap test (ASC or higher), while 80 had a normal Pap test. The comparison group includes

270 women who had a positive HR-HPV test at baseline and who cleared their infection during the follow-up [21]. Among these women, the baseline Pap smear was abnormal in 180 and normal in 90 cases. In all subsequent analyses, Group 1 (n = 273) was compared with Group 2 (n = 270), to disclose the variables explaining persistence and non-persistence (= clearance) of HR-HPV infections, respectively.

Defining persistent cases and viral clearance

In all women, a minimum of two HPV tests were done. As a persistent infection, we regarded all cases with a positive HPV test for HR types at 1 pg/ml RLU/CO cut-off at baseline and at their last visit, and who did not have any HPV-negative test during the follow-up [28]. Patients who tested HPV-negative between two HPV-positive tests were considered as having a fluctuating infection, and they were excluded from the present cohorts. As viral clearance, we considered all baseline HPV-positive women, who developed a negative HCII test during the follow-up, and who remained HPV-negative at their last visit. Cases who tested HPV-positive subsequent to an HPV-negative test were also considered as fluctuating infections, and were not included in the study

Methods

Epidemiological Questionnaire

At the first visit all women who agreed to participate in the study responded to a detailed inquiry recording the implicated and suspected risk factors of cervical cancer (CIN and HPV), altogether 66 items including reproductive history, sexual behaviour and smoking habits. The detailed analysis of the collected epidemiological data was reported recently [32].

Papanicolaou (Pap) Smears

All women were subjected to a cervico-vaginal Papanicolaou smear, interpreted using the jointly agreed terminology (modified Papanicolaou classification), as previously described [31]. For statistical purposes, this classification was transformed to correspond to the Bethesda 2001 system [12, 21, 31]. The primary screening and interpretation of the smears were done in the NIS clinics, and all slides were subjected to re-screening by two IAC-certified cytotechnologists and interpretation by one cytopathologist (FIAC) in Finland [31].

Detection of HPV DNA

The sample for the Hybrid Capture II test was taken from the cervix using the HCII sampling kit (Digene, Silver Springs, MD, USA). All samples were delivered to Turku, Finland, within two weeks to cope with the manufacturer-guaranteed test validity period of 14 days from the sampling. The test was performed according to the provider's instructions using the probe panel B which detects high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. The RLU/CO value of 1 pg/ml (approximately 8,000 copies of HPV/test) was used as the cut-off for a positive test [12, 21, 31, 33].

PCR amplification

PCR analyses were done from the samples collected for HCII assay, using the Digene Cervical Sampler [12, 31, 33]. These samples were processed for DNA extraction with the high salt method of Miller et al. [34]. HPV DNA was detected with PCR using GP05+/GP06+ primers. Confirmation of the specificity of PCR products was done by hybridization with digoxigenin-labeled HR-HPV (with types 16, 18, 31, 33, 35, 39, 45, 51, 52, 54, 56 and 58) oligoprobes, as described recently [35, 36]. After hybridisation, the positive spots in the film were graded (semi-quantitatively) according to the signal intensity, as 1) negative, 2) weak, 3) moderate, or 4) strong.

The analytical sensitivity of our PCR method is approximately 20 copies of HPV, i.e., 20 SiHa cells mixed with 300 ng of human fibroblast DNA give a strongly positive signal [35]. For evaluation of the possible contamination during DNA extraction, DNA was simultaneously extracted from cultured human fibroblasts. Additionally, every eighth sample for PCR contained no DNA. DNA dilution of SiHa cells was used as a positive control for HPV DNA detection [35, 36]. DNA extraction, master mix for PCR, and adding of target DNA in the reaction mixture were all done in separate rooms.

Statistical analyses

Statistical analyses were performed using the SPSS, for Windows program package (version 11.5). Frequency tables were analysed using the Chi-square test, and the likelihood ratio (LR) statistics was used to assess the correlation between the categorical variables. Differences in the means of continuous variables between the groups were analysed using non-parametric tests (Mann-Whitney) or ANOVA (analysis of variance) test, after careful control of the normal distribution (Kolmogorov-Smirnov test with Lilliefors correction). Logistic regression models were used to analyse the power of different variables as predictors of the outcome variables (persistence, clearance) both in univariate- (crude ORs and 95% CI) and multivariate (adjusted ORs and 95% CI) analysis, using the stepwise backward approach and LR (likelihood ratio) statistic for removal testing ($p = 0.10$ probability for stepwise removal, and $p = 0.05$ probability for stepwise entry). ROC curve analysis was used to assess the sensitivity and specificity of HCII in detecting the outcome measure (HPV persistence), and also the positive predictive value (PPV) and negative predictive value (NPV) were calculated using the contingency tables. In all tests, the values $p < 0.05$ were regarded as statistically significant.

Results

The baseline epidemiological, clinical and virological data recorded from the patients and their samples were compared in the two series of women, as shown in Table 1. Women with persistent infections were on average two years younger than those who cleared their infection. The length of follow-up was significantly longer for those who cleared

Table 1. — *Baseline data of the women with persistent and cleared high-risk HPV infection.*

	Persistence / Clearance		
Characteristics	Persistent HR	Cleared HR	Significance
Number of patients:	273	270	
Age (M±SD)	27.3 ± 9.3	29.1 ± 9.3	p = 0.006*
Follow-up time (M±SD)	14.1 ± 10.7	21.0 ± 10.8	p = 0.0001*
Patient Category:			p = 0.025
Screening patient	113 (41.4%)	125 (46.3%)	
Gynaecological patient	52 (19.0%)	67 (24.8%)	
STD patient	108 (39.6%)	78 (28.9%)	
Age at menarche (M±SD)	13.4 ± 1.4	13.2 ± 1.4	p = 0.375*
Periods regular:	189 (84.4%)	199 (85.4%)	p = 0.795
Ever pregnant:	167 (66.8%)	176 (69.8%)	p = 0.502
Ever miscarriages:	28 (11.8%)	23 (9.4%)	p = 0.460
Ever abortions:	123 (52.1%)	128 (52.2%)	p = 1.000
Onset of sexual activity:	18.9 ± 2.8	18.8 ± 2.8	p = 0.825*
Sexual activity regular:	113 (46.9%)	123 (51.0%)	p = 0.412
Intercourses per week:	3.6 ± 3.5	3.6 ± 3.4	p = 0.953*
Intercourses per week during the past 2 years:	2.4 ± 2.7	2.4 ± 2.4	p = 0.854*
Currently one partner only:	213 (85.9%)	206 (82.4%)	p = 0.327
No. of partners (past 2 years:)	2.0±2.5	1.8±2.4	p = 0.307
Partners with genital warts:	25 (10.2%)	28 (11.4%)	p = 0.459
Ever had any STD:	40 (16.3%)	48 (19.8%)	p = 0.347
Practice oral sex:	123 (54.7%)	137 (59.8%)	p = 0.297
Practice anal sex:	29 (13.6%)	19 (9.6%)	p = 0.222
Casual sexual partners:	43 (17.6%)	43 (17.8%)	p = 1.000
Domestic casual partners:	29 (51.8%)	34 (54.0%)	p = 0.855
Casual partners abroad:	5 (3.4%)	10 (7.1%)	p = 0.192
Use contraception regularly:	122 (50.4%)	138 (57.0%)	p = 0.171
Bide/douche at intercourse:	224 (93.3%)	228 (93.8%)	p = 0.855
Request B/D from partners:	204 (84.0%)	206 (84.8%)	p = 0.901
Ever had warts (skin, oral, anal):	71 (29.2%)	66 (26.9%)	p = 0.615
Ever had CIN detected/treated:	15 (7.3%)	21 (10.0%)	p = 0.385
Ever taken Pap smear:	77 (37.7%)	77 (36.3%)	p = 0.839
Previous Pap test normal:	54 (71.1%)	55 (64.7%)	p = 0.404
Ever had radiotherapy:	5 (2.2%)	5 (2.2%)	p = 1.000
Anti-diabetics (oral or insulin):	2 (0.9%)	2 (0.9%)	p = 1.000
Use of cortisone:	1 (0.4%)	3 (1.3%)	p = 0.371
Use of chemotherapeutics:	3 (1.3%)	2 (0.9%)	p = 1.000
Any chronic illness (non-gynaecological):	86 (36.1%)	69 (30.0%)	p = 0.170
Current regular smoker:	81 (32.5%)	63 (25.6%)	p = 0.055
Years being regular smoker:	6.7 ± 5.4	7.9 ± 5.8	p = 0.202
No. of cigarettes per day:	7.7 ± 4.9	8.9 ± 4.8	p = 0.136
Ever been regular smoker:	24 (14.4%)	40 (22.3%)	p = 0.071
Ex-smoker: years being smoker:	6.0 ± 4.3	4.2 ± 3.8	p = 0.119
Ex-smoker: No. of cigarettes per day:	6.4 ± 3.2	5.7 ± 4.9	p = 0.604
Partner being current smoker:	139 (58.9%)	141 (59.5%)	p = 0.926
Ever had cervical erosion:	154 (62.3%)	148 (60.4%)	p = 0.711
Time since erosion detected:	7.2 ± 7.9	7.3 ± 7.3	p = 0.927
Cervical erosion treated:	77 (40.1%)	93 (48.9%)	p = 0.099
Mode of treatment:			p = 0.311
Conservative	38 (46.3%)	36 (38.7%)	
Electrocoagulation	43 (52.3%)	53 (57.0%)	
Cryotherapy	1 (1.2%)	4 (4.3%)	

the HPV. Of the women with persistent HPV, almost 40% were STD patients (p = 0.025). All the 66 variables recorded by the questionnaire [32] were compared in the two groups. Only being a current smoker reached borderline statistical significance, p = 0.055.

Colposcopy, baseline Pap test result or the first cervical biopsy did not predict HR-HPV persistence, whereas disease outcome monitored by the Pap test was markedly different in the two groups (p = 0.0001). Viral load (RLU/CO) assessed by the HCII test was significantly higher among persistent infections, as was the proportion of cases with RLU/CO values above the 100 cut-off value (p = 0.0001). Similarly, being HR-HPV positive with PCR was significantly more frequent among the women whose HPV remained persistent, while the semi-quantitative grading of the PCR reaction was of no significance.

The results of univariate regression analysis are shown in Table 2. Both age and follow-up time had a "protective effect" against persistence, i.e., older patients and those with longer follow-up had a lower probability of having persistent HPV. Being an STD patient slightly increased the probability of persistence (OR 1.532, 95% CI 1.040-2.255). Disease outcome monitored by the Pap test was a significant predictor of persistence, particularly two of the outcomes: incident abnormal Pap test and persistent abnormal Pap test. Similarly, high viral load and HR-positive PCR were significant predictors (p = 0.001 and p = 0.0001, respectively).

In the multivariate regression model, all these variables were controlled for the confounding effect of age and follow-up time to calculate the adjusted OR (95% CI). Patient category and baseline Pap test lost their significance. On the other hand, being a current smoker proved to be significant (p = 0.014), and thus negatively confounded by age and follow-up time. The rest of the significant predictors maintained their significance as independent predictors in this multivariate model.

When all significant variables were entered in the final model, only two of them remained independent predictors of HR-HPV persistence: follow-up time (p = 0.0001) and positive PCR for HR-HPV (p = 0.001). Follow-up time had a protective effect on persistence (OR 0.950, 95% CI 0.924-0.976). Being HR positive in PCR was a powerful predictor of persistence, OR 4.169 (95% CI 1.74-9.987). Although, Pap test outcome as a whole was not an independent predictor, an incident abnormal Pap test was a significant predictor, with OR 4.291, (95% CI 1.057-17.419; p =

Table 1. — *Continued.*

Characteristics	Persistence / Clearance		
	Persistent HR	Cleared HR	Significance
Partner being current smoker:	139 (58.9%)	141 (59.5%)	p = 0.926
Ever had cervical erosion:	154 (62.3%)	148 (60.4%)	p = 0.711
Time since erosion detected:	7.2 ± 7.9	7.3 ± 7.3	p = 0.927
Cervical erosion treated:	77 (40.1%)	93 (48.9%)	p = 0.099
Mode of treatment:			p = 0.311
Conservative	38 (46.3%)	36 (38.7%)	
Electrocoagulation	43 (52.3%)	53 (57.0%)	
Cryotherapy	1 (1.2%)	4 (4.3%)	
Baseline Pap smear abnormality:			p = 0.058
N-SIL	163 (60.4%)	191 (71.0%)	
ASC	36 (13.3%)	28 (10.4%)	
LSIL	64 (23.7%)	47 (17.5%)	
HSIL	7 (2.6%)	3 (1.1%)	
Pap Test Outcome:			p = 0.0001
Always negative	23 (8.5%)	43 (16%)	
Incident abnormal	49 (18%)	43 (16%)	
Persistent abnormal	125 (46%)	78 (29%)	
Cleared abnormal PAP	64 (23.5%)	96 (35.7%)	
One abnormal test only	7 (2.6%)	6 (2.2%)	
Fluctuating ±	4 (1.5%)	3 (1.1%)	
Significant colposcopy@:	36 (17.3%)	26 (12.3%)	p = 0.170
First cervical biopsy:			p = 0.277
Normal	40 (28.0%)	54 (35.1%)	
HPV-NCIN	41 (28.7%)	29 18.8%)	
CIN I	34 (23.8%)	40 (26.0%)	
CIN II	16 (11.2%)	20 (13.0%)	
CIN III	11 (7.7%)	10 (6.5%)	
HPV viral load in HCII (RLU/CO):	492.4 ± 688.6	307.1 ± 577.6	p = 0.0001*
High/low viral load (RLU/CO 100 cut-off):	146 (53.5%)	94 (34.8%)	p = 0.0001
PCR result (HR +) (n=289):	118 (90.8%)	116 (73.0%)	p = 0.0001
PCR results (Graded):			p = 0.067
Negative	23 (17.7%)	33 (20.8%)	
Weak positive	36 (27.7%)	60 (37.7%)	
Intermediate positive	42 (32.3%)	31 (19.5%)	
Strong positive	29 (22.3%)	35 (22.0%)	

*Mann-Whitney; @leukoplakia, coarse mosaic, coarse punctation; #logarithmic scale.

0.042), whereas the other outcomes were not significant.

Discussion

Our previous analysis of the epidemiological data collected from the entire cohort of 3,187 women disclosed the well known risk factors of HR-HPV among these women tested for optional screening tools in three New Independent States (NIS) of the former Soviet Union [32]. In the present study, an attempt was made to disclose whether any of these epidemiological factors would predict the persistence of HR-HPV infections in these women. Women with persistent infections were two years (mean) younger than those who had their infection cleared. This is consistent with our previous data analysing the acquisition and clearance of HR-HPV infections [12, 21]. The different age-specific incidence- and clearance rates explain the differences in the age-specific prevalence of HR-HPV infections in this study population. Because these clearance events progress with a relatively constant monthly rate [21], many of the currently HPV-positive women are likely to clear their infection during the next two years, which is the age difference between the two groups in this series. This difference is well illustrated by the longer follow-up time of the women who cleared their HPV as compared with the persistors (Table 1). Thus, age and the length of follow-up time are important confounding factors to be controlled in the multiple regression analysis of these data.

Of the 66 data variables recorded by questionnaires, only being a current smoker was different in the two series (p = 0.055). Our analysis could not confirm e.g. the role of oral contraception as a determinant of HPV persistence, as reported in some previous studies [37, 38]. Our data are more consonant with that of Ho *et al.* (1995), who did not find any association between the use of oral contraceptives and persistent HR-HPV [39]. The role of smoking as a predictor of HPV persistent is highly interesting however [3-5, 7, 8, 14, 30]. In univariate analysis, being a current smoker (but not ex-smoker) reached borderline significance (OR 1.401, 95% CI 0.948-2.069) as a predictor of HPV persistence. Unexpectedly, when adjusted for the confounders (age and length of follow-up), smoking status was negatively confounded and became an independent predictor (Table 2) with OR 1.693 (95% CI 1.114-2.573; p = 0.014). Thus, if adjusted by age and follow-up time, current smokers have a 69% increased risk of their HR-HPV becoming persistent. There are studies failing to disclose any such association [28, 39, 40], while many more provide data substantiating our present observations [37, 41-44] and the concept of smoking as a potential risk factor of cervical cancer [3, 4, 7, 14, 45]. Thus, women smokers might need closer surveillance of their HPV infections than non-smokers because of this increased risk of remaining persistent.

This failure to detect any significant predictors among these questionnaire-collected data items implicates that recording such extensive epidemiological data is likely to be of limited value in identifying the women at increased risk for persistent HR-HPV. In the same way, this risk cannot be accurately predicted by the findings in the baseline colposcopy or baseline Pap test. On the other hand, monitoring the patient by repeated Pap smears is an accurate means to predict

persistent HR-HPV infection, as shown before [13, 27, 37, 39]. A feasible explanation is that viral acquisition and clearance are temporarily closely related to the clinical course of the disease monitored by the Pap test [12, 21, 33]. When adjusted for the confounders, an incident abnormal Pap (OR 3.028, 95% CI 1.516-6.048) and persistent abnormal Pap (OR 2.919, 95% CI 1.595-5.341) were independent predictors of HR-HPV infection. In the model with all factors entered, the predictive power of an incident abnormal Pap increased even further, with OR 4.291, (95% CI 1.057-17.419; p = 0.042). The practical implication of these data is that monitoring women with the Pap test is an adequate means to monitor persistent HR-HPV, and if properly done, is a safeguard against the development of cervical cancer, as shown by the organised Pap smear-based screening programmes [3, 4, 7].

The role of viral factors (HPV polymorphisms, integration, viral load, etc.) as determinants of HPV persistence have been studied to some extent before [5, 8, 9, 14, 15, 22-24, 28, 30]. In the present study, women with persistent HR-HPV showed significantly higher viral loads (RLU/CO) in their baseline sample (p = 0.0001) as compared to those who cleared their infection (Table 1). In ROC analysis for HCII as a predictor of persistence, the area under the ROC curve was 0.622 (95% CI 0.575-0.669) (p = 0.001). The best balance between sensitivity and specificity (57.5% and 60.0%) was obtained with the RLU/CO cut-off 71.3 pg/ml. The RLU/CO cut-off 100 pg/ml was a significant predictor of persistence, with OR 2.152 (95% CI 1.524-3.040) (p = 0.0001), and sensitivity 65.1%, specificity 53.4%, PPV 58.1%, NPV 60.8%. The high viral load maintained its statistical power as an independent predictor of persistent HR-HPV in multivariate analysis adjusted for age and follow-up time (Table 2). These data are consonant with the recent reports implicating that the high viral load of oncogenic HPV types is an important risk factor for persistent infections and progressive clinical disease [3, 4, 7, 14, 23, 39, 46-49]. This notwithstanding, however, it should be emphasised that the length of follow-up time has a significant impact on disease outcome, as shown by our 20-year prospective follow-up study in Finland [50]. In the present study the predictive value of high viral load did not overcome that of the follow-up time when all significant variables were entered in the regression model. This indicates that by far not all high viral load HPV infections that are shown to persist for a relatively short follow-up time (e.g. 14 months in this case) remain persistent for longer periods. This was well documented in our recent studies on HPV dynamics showing e.g. that virus clearance precedes the disappearance of the lesion by two to three months [12, 21, 33].

One of the most powerful independent determinants of HPV persistence was the HR-HPV positive PCR at baseline, which maintained its power even when all significant variables were entered in the multivariate regression model, with OR 4.169 (95% CI 1.741-9.987) (Table 1 and 2). As discussed before, high viral load lost its predictive power in this model. This somewhat unexpected observation is explained by the differences between PCR and HCII, and their imperfect concordance. In addition to us [36], the issues related to viral load measurements

Table 2. — *Factors predicting HR-HPV persistence in univariate and multivariate regression analysis.*

	Univariate analysis		Multivariate analysis	
Variable	Crude OR (95% CI)	Significance	*Adjusted OR (95% CI)	Significance
Age	0.979 (0.961-0.998)	p = 0.027		
Follow-up time	0.944 (0.928-0.959)	p = 0.0001		
Patient category:		p = 0.026		p = 0.409
Screening	Reference		Reference	
Gynaecologic	0.859 (0.551-1.337)		1.324 (0.870-2.014)	
STD	1.532 (1.040-2.255)		1.068 (0.665-1.718)	
Current smoker	1.401 (0.948-2.069)	p = 0.055	1.693 (1.114-2.573)	p = 0.014
Baseline PAP smear		p = 0.063		
NSIL	Reference		Reference	p = 0.102
ASCUS	1.507 (0.881-2.576)		1.180 (0.666-2.091)	
LSIL	1.596 (1.037-2.454)		1.502 (0.952-2.367)	
HSIL	2.734 (0.696-10.744)		4.056 (0.957-17.200)	
Pap Test Outcome:		p = 0.0001		p = 0.005
Always negative	Reference		Reference	
Incident abnormal	2.130 (1.111-4.086)		3.028 (1.516-6.048)	
Persistent abnormal	2.996 (1.678-5.351)		2.919 (1.595-5.341)	
Cleared abnormal PAP	1.246 (0.686-2.264)		1.769 (0.942-3.324)	
One abnormal test only	2.181 (0.655-7.258)		1.824 (0.525-6.342)	
Fluctuating ±	2.493 (0.513-12.105)		4.054 (0.793-20.727)	
HPV viral load (HCII)	1.000 (1.000-1.001)	p = 0.001	1.000 (1.000-1.001)	p = 0.006
High vs low viral load (RLU/CO 100 cut-off)	2.152 (1.524-3.040)	p = 0.0001	2.012 (1.402-2.888)	p = 0.0001
PCR result (HR ±)	3.645 (1.830-7.262)	p = 0.0001	4.085 (2.006-8.320)	p = 0.0001
PCR result (Graded):		p = 0.070		p = 0.235
Negative	Reference		Reference	
Weak positive	0.861 (0.439-1.689)		0.871 (0.435-1.741)	
Intermediate positive	1.944 (0.959-3.939)		1.672 (0.806-3.468)	
Strong positive	1.189 (0.576-2.455)		1.210 (0.573-2.553)	

*adjusted for age and length of follow-up time.

by HCII and PCR have been recently addressed by other authors as well [51, 52]. While Gravitt *et al.* demonstrated that viral load measurements by HCII in the presence of multiple HPV infections overestimated type-specific viral load [51], Pretet *et al.* (2004) showed that the HCII values can be used as a quantitative measure of HR-HPV DNA, provided that cervical specimens are collected using standardised protocols [52]. This was the case in our study, where the DNA samples for both PCR and HCII were collected using the same samplers, amplified by PCR [35, 36, 48].

By definition, all 543 women included in the two series tested HR-HPV positive with HCII at baseline. However, only 90.8% of persistent infections and 73.0% of those who cleared their HR-HPV were HR-HPV positive at baseline PCR ($p = 0.001$). This is consonant with our recent comparison of these two techniques in a series of 1,500 samples, showing that HCII and PCR are concordant in about 85% and the kappa values fall within the "substantial agreement" category [36]. Our data are in alignment with the recent observations of Ho *et al.* [39], who showed that women with PCR-positive HR-HPV infection (with high viral load) had the highest risk for persistent disease. According to our data, positive PCR for HR-HPV is an independent predictor of persistent infection, exceeding even the statistical power of viral load determined by HCII assay.

Taken together, these data neatly fit with our recent concepts on HPV evolution in cervical carcinogenesis, starting from purely episomal infections, progressing to mixed forms and finally ending up with a purely integrated form in high-grade CIN and cancer [53, 54]. Clearly, the infections with a mixed episomal/integrated HPV DNA state are capable of regression, as shown by the present data. Thus, only a minor fraction of integrated HPV16 and other HR infections will finally end up as CIN3 and cervical cancer, and the well established HPV type distribution in the latter [3, 4, 6, 7] merely reflects their relative frequencies in precancerous lesions rather than their different risks for persistent infections, as detected during the short follow-up time of the present study.

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Address reprint requests to:
Prof. K. SYRJÄNEN, M.D., Ph.D., FIAC
Department of Oncology & Radiotherapy
Turku University Central Hospital
Savitehtaankatu, 1 - Room B 419
FIN-20521 Turku (Finland)